ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 27. SUPPLEMENTUM 97

Active transport of ions through frog skin with special reference to the action of certain diuretics

A study of the relation between electrical properties, the flux of labelled ions, and respiration

BY

HÅKAN LINDERHOLM

UPPSALA 1952

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AKADEMISK AVHANDLING

SOM MED TILLSTÅND AV MEDICINSKA FAKULTETEN VID UPPSALA UNIVERSITET FÖR ERNÅENDE AV MEDICINE DOKTORSGRAD OFFENTLIGEN FÖRSVARAS Å FYSIOLOGISKA INSTITUTIONENS HÖRSAL LÖRDAGEN DEN 24 MAJ 1952 KL. 10 F. M.

AV

HÅKAN LINDERHOLM
MED. LIC., SMÅL.

UPPSALA 1952
APPELBERGS BOKTRYCKERI AB

R. UNIV.
BIBLIOTHEEK
LEIDEN

ACTA PHYSIOLOGICA SCANDINAVICA
VOL. 27. SUPPLEMENTUM 97

From the Physiological Institute, University of Uppsala, Sweden

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List of symbols.

A	area.
a	activity (chemical).
$\boldsymbol{a}$	total activity.
B	a chemical component.
c	concentration.
D	diffusion coefficient.
F	Faraday (96 500 coulombs).
f	activity coefficient (in the molar scale).
G	total electrical conductance.
G^i	partial conductance of the ion B^i. For definition see eqn. (1.3.3).
$G^{Na}_{a\,I}$	and $G^{Na}_{a\,II}$ are the partial conductances of the actively transported Na ions calculated in two different ways from the experimental data (see 2.3.B, p. 57).
I	electric current.
M	molar (moles/liter).
n	recorded counting rate in counts per minute.
N	true counting rate or relative activity of radioactive isotopes in counts per minute.
N_0	relative activity at zero time.
N_t	relative activity at a time t.
Q_{O_2}	μl O_2 taken up per mg dry weight of tissue per hour.
q	pipette volume.
R	gas constant.
T	absolute temperature.
Δt	time interval.
z	electrovalency of an ion (positive for cations, negative for anions).
v	volume.
X	»effective sodium carrier concentration» (cf. 2.3.D.c).
Φ	net flux ($\Phi = \Phi_1 + \Phi_2$) (for definition cf. 1.2.a and 1.3.a).
Φ_1	total flux from phase 1 to phase 2.
Φ_2	total flux from phase 2 to phase 1.
ψ	is defined by eqn. (1.3.10).
φ	electrical potential ($\varphi = \varphi_2 - \varphi_1$).
φ_a	active transport potential.

$\varphi_{a\mathrm{I}}^{+}$, $\varphi_{a\mathrm{II}}^{+}$, $\varphi_{a\mathrm{I}}^{\mathrm{Na}}$ and $\varphi_{a\mathrm{II}}^{\mathrm{Na}}$ are active transport potentials of positive ions, respectively sodium ions, calculated in different ways from the experimental data (see 1.7).

ξ^{z^i} = exp. $\{(\varphi_2 - \varphi_1)\, z^i F/RT\}$, cf. eqn. (1.2.2), is the contribution of the electrical potential to the total activity of an ion.

ξ_a is defined by eqn. (1.2.7) and describes the contribution of the active transport potential to the total activity of the ion.

ξ_a^w is defined by eqn. (1.8.3) and describes the contribution of the active transport of water to the total activity of water.

ξ_w^i is defined by eqn. (1.9.2) and (1.9.3) and describes the contribution of the streaming of water to the total activity of the ion.

ln natural logarithm ($\log_e$).

log logarithm to base 10 ($\log_{10}$).

Superscripts refer to component species as follows:

i refers to an ionic species.

+ and — refer to positive and negative ions respectively.

Cl, K, and Na refer to chloride, potassium, and sodium ions respectively.

w refers to water.

Subscripts refer to phases or solutions on either side of a membrane or to actively and passively transported components as follows:

1 refers to phase 1, in the case of frog skin always the epithelial side (outside).

2 refers to phase 2, in the case of frog skin always the chorium side (inside) of the skin.

a, ***a*** are used to denote active transport and symbols connected with actively transported components. For the meaning of ***a***, see 1,6.a.

p refers to passive ions.

Introduction.

The mercurial diuretics and theophyllamine, according to the generally accepted view, exert their main diuretic action by decreasing the reabsorption of sodium chloride from the kidney tubules, which in its turn decreases the reabsorption of water. We know practically nothing about the details of their action mechanism. We are similarly ignorant about the mechanisms by which the active reabsorption of electrolytes and water takes place through the renal tubular epithelium.

An analysis of these processes in the complex tissue of the kidney is difficult, and it was thought necessary to begin by examining a simpler system. Isolated frog skin is convenient for this purpose, and has already been thoroughly investigated in many respects in model experiments. It is known to transport sodium chloride and water from the outside to the inside. In this respect it has a similar function to the epithelium of the renal tubules. The action of diuretics on frog skin may thus resemble in some ways their action on the renal tubular epithelium.

Substances such as sodium chloride may be transported through the tubular epithelium or through frog skin from a solution in which the transported substance has a low concentration to a solution where the concentration is high, as well as from one solution to the other when the concentration is the same on both sides. As a rule, both these processes require the expenditure of energy in the membrane.

This investigation was undertaken to study such transport phenomena in frog skin, with particular regard to the effects of the diuretic drugs mentioned. The active transport of ions is analyzed by the combined use of electrical and radioactive tracer methods and the efficiency of the transport process is estimated from measurement of oxygen uptake.

Chapter 1.

Theoretical considerations regarding the active transport of ions.

1.1. Active transport and its definition.

It is not the purpose of the present paper to give a complete review of the extensive literature concerning active transport. The field was recently reviewed by KROGH (1946) and USSING (1949 a). HÖBER (1945) also gives a good survey of the problems involved. The thermodynamic aspects were recently discussed by ROSENBERG (1948).

The active transport of substances is of great importance in biology, and is intimately connected with the problems of how osmotic or hydrostatic work is performed by living cells. The ability to do this work enables living cells and multicellular organisms to maintain a composition of their fluids with respect to electrolytes which differs a great deal from the surrounding medium or from substances or fluids which the organisms use as food. (cf. H. W. SMITH 1943, KROGH 1946, CONWAY 1947). The maintainance of such a state requires a continuous expenditure of energy.

With regard to the exact details of the mechanism by which this work is performed, we know very little, whether it occurs in a cell membrane, in the gills of fishes, in the skin of amphibians, in the gastric or intestinal mucosa, in the secretory glands, or in the kidneys. Many possible mechanisms have been put forward to account for the osmotic work and the transport of electrolytes. They all require an energy-delivering (chemical) reaction which is linked by the mechanism proposed to the transport of the substance across the membrane. A carrier substance is often postulated which takes part in the reactions and to which the actively transported substance is specifically bound. Some of the mechanisms that have been proposed will be briefly described.

Osterhout and Stanley (1932) described a model which was able to accumulate potassium by means of a carrier to which potassium was selectively bound, and which could imitate the behaviour of the *Valonia* cell in many respects (cf. also Osterhout 1940). The theory of the diffusion in this cell was discussed by Longsworth (1933). The model is instructive though it does not involve active transport in the sense used here (see below). The same can be said about the accumulation of ions in the diffusion model described by Teorell 1935, 1937). A difference in the electrochemical potential of the ions in the two solutions on either side of the membrane is the force which produces the accumulation.

A mechanism of wide general applicability for accomplishing active transport has been proposed by Frank and Mayer (1947). They call it an »osmotic diffusion pump« and it makes possible the transport of both solvent and solutes on a basis of certain simple assumptions. They also calculated the thermodynamic efficiency of the pump and found it to be about 30 per cent under favourable conditions. This mechanism may also be used to explain, for instance, the absorption of glucose with the aid of phosphorylation processes (Verzar 1931, Wilbrandt and Laszt 1933).

A difference in the oxidation reduction potential across the membrane, maintained by the metabolism of the cell, has also been used in many theories to explain the force behind the transport mechanism. The first theories (Lund 1928, Stiehler and Flexner 1938), were unsatisfactory as they required the movement of free electrons, which introduces a difficulty when dealing with electrolyte solutions. Lundegårdh (1940, 1947, 1948, 1949) presented much evidence for the conclusion that anions are actively transported across root hairs of plants by a redox mechanism in which the movement of free electrons is not required. Cytochrome molecules which constitute the redox system in this theory are assumed to exchange electrons by rotation inside the membrane. Lundegårdh's theory was further developed by Eriksson (1949) who combined it with diffusion conditions imposed by charged membranes. Little specificity is associated with such anion transport.

Conway (1951), in a further development of the redox theory, described a possible redox pump mechanism which transports cations specifically and with high efficiency by means of specific adsorption of the ions on a metal catalyst. A similar transport of anions is stated to be possible. In view of the general occurrence in biological systems of the redox processes required by the pump described, the mechanism seems quite plausible. It also makes it possible to relate active transport to respiration.

With regard to respiration, there seem to be two kinds of mechanisms for active transport of ions, those which work aerobically and those which depend on glycolysis. Thus the active extrusion of sodium from red blood corpuscles (and muscle?) seems to depend on glycolysis (Maizels 1951), while the transport of sodium through frog skin (Huf, Ussing) depends on an aerobic process which will be discussed later. The same seems to be true for the active transport of sodium in the kidneys (Conway, FitzGerald and MacDogald 1947), and for anion transport through plant roots (Lundegårdh 1940).

In the case of frog skin, Ussing (1949 b) proposed a possible mechanism for the active transport of sodium which will be considered later.

This paper will not deal so much with the nature of the pump mechanism by which the osmotic work is performed, but mainly with the result of its work (determined from changes in the composition of the solutions on both sides of the membrane). This is controlled by the working conditions which the membrane as a whole offers the »pump».

It is felt that there is too little known about the conditions which govern the active transport and factors that influence it to allow definite conclusions concerning the nature of the »pump . Only an extended study of many factors which influence a certain system will make it possible to draw more detailed conclusions about the transport mechanism. As already mentioned, several factors are known to be of importance. In the case of the frog skin, on which our interest will be especially focussed, many facts have been assembled concer-

ning the effect of the composition of the bathing fluids, hormones, and several enzyme poisons on the active transport of salt (see below). More facts, however, are required in order to elucidate the transport mechanisms. Some diuretics are known to have a marked influence on the transport of electrolytes in the kidney. Their effect on the active transport of salt through frog skin will be studied in this investigation.

According to ROSENBERG (1948), active transport is a transfer of matter which for energetic reasons cannot take place spontaneously, its performance requiring coupling to compensating processes. He then defines active transport as a transfer of matter from a lower to a higher chemical (in the case of charged components: electrochemical) potential. This definition seems too narrow. It should be added that other potentials due to differences in temperature and pressure are assumed not to exist together with the electrochemical potential, but even then it might seem arbitrarily chosen if, for instance, an oxidation reduction potential gradient through a membrane should be excluded from the other energy forms not included in the active transport. From this point of view the transport of an ion by means of the redox pump described by CONWAY (1951) is no more active than the accumulation of ions in the models of OSTERHOUT and TEORELL. However, CONWAY's mechanism presumes a certain structure in the membrane as well as enzymatic reactions in or at the membrane. Such enzymatic reactions are also assumed in the »osmotic diffusion pump« and in other carrier mechanisms proposed. We will therefore attempt to *define active transport* as transport due to forces or potentials of any kind which depend on enzymatic reactions in or at the cell membranes maintained by the continuous metabolic processes of the cells, and which make possible a transfer of matter from a lower to a higher (electro)chemical potential — other potentials such as those due to differences in temperature or pressure or the streaming of solvent being absent. If all these last mentioned potentials and the (electro)chemical potential are included in a *total »non-active« potential* this term could replace the electrochemical potential in the definition. Further, the *total potential*

could be defined as the sum of all active and non-active potentials across the membrane, in agreement with the general concept applied by TEORELL (1949 a, 1950). A more general definition of active transport, including also the case when active transport occurs in the same direction as the transport due to the electrochemical potential, is given later in this chapter by eqns. (1.2.7), (1.8.3) and (1.9.4) in which the active transport force is defined by the rates of transfer.

Some criticism may be raised against the term »active transport» as such. It may seem to allude to some »vital activity» or »intelligence» of the cells which was long associated with words such as secretion or excretion (cf. SMITH 1937). The term active transport is now generally accepted, however. Defined as above, active transport has no implication of »vital activity», but is regarded as a link in the chain of thermodynamically irreversible reactions involved in the metabolism of the cells which maintains the dynamic equilibrium state of the cells that is characteristic of life. In this connection it should be remarked that thermodynamics in the classical form cannot be applied to a system like a living cell (HAASE 1951), which must be regarded as a thermodynamically open system continuously exchanging energy and matter with its surroundings.

1.2. Flux of ions through membranes with and without active transport.

In the following sections of this chapter some theoretical considerations will be formulated with the aim of making possible a more or less quantitative treatment of data obtained in experiments on isolated frog skin with the combined use of electrical measurements (of potential and conductance) and tracer methods. The experiments were made in order to study the active transport of electrolytes through the skin.

Some simplifying assumptions with regard to the experimental conditions, which are discussed later in 2.3.A and 2.3.D, will be made to make it possible to render the treatment in a simple mathematical form.

a. *Membranes without active transport.* A system will be considered in which two solutions of electrolytes in water,

1 and 2, of the same temperature and at the same hydrostatic pressure, are separated by a membrane. It is assumed that there is no streaming of water through the membrane. The two solutions are assumed to be ideally stirred (or the diffusion across the membrane to be slow as compared with diffusion in water). When a stationary state has been established, any ion which is not actively transported — in the following often referred to as »passive ions» — has been shown to fulfil the condition

$$- \Phi^i_2 / \Phi^i_1 = a^i_2 \, \xi^{z^i} / a^i_1 \quad . \tag{1.2.1}$$

where Φ^i_1 means the rate of passage or the flux of the ion B^i from phase 1 to 2, i.e. the total quantity of the ion B^i which crosses unit area of the membrane in direction 1 to 2 in unit time. $-\Phi^i_2$ represents the flux from 2 to 1. a^i_1 is the activity of the ion B^i in solution 1 and a^i_2 that in solution 2. ξ^{z^i} is defined by (cf. PLANCK 1890, TEORELL 1951)

$$\xi^{z^i} = \exp\left[(\varphi_2 - \varphi_1)\, z^i F / R T\right] \text{ or } \varphi = \varphi_2 - \varphi_1 = (R T / F) \ln \xi \,^{1} \tag{1.2.2}$$

and represents the contribution of the electrical potential to the electrochemical activity of the ion (»electrochemical activity coefficient», TEORELL). φ_1 and φ_2 are the electrical potentials of solution 1 and 2. z^i is the valence of the ion of the i-th species, positive for a positive and negative for a negative ion. N.B. z^i is the power of ξ). R is the gas constant, T is the absolute temperature and F is the Faraday.

Eqn. (1.2.1) was derived by USSING (1949 c) for use in work on frog skin. If the activity coefficients are disregarded, the same formula may be derived from a flux relation given by BEHN 1897 (TEORELL 1949 b), and it appears as a special case

[1] It should be remarked that the ξ^{z^i} as defined by (1.2.2) may have another meaning than the ξ used by TEORELL (1951). As no assumption concerning the structure of the membrane has to be made in connection with eqn. (1.2.1), it may be a charged membrane. In that case the Donnan potentials are included in the total potential difference, and the ξ^{z^i} used here then corresponds to $\xi \, r_2 / r_1$ in TEORELL's notation.

in a generalized theory of membrane permeability (TEORELL 1951). The formula is quite general with the restrictions mentioned above, and the nature of the membrane is of no importance for its validity, with one exception:

If »exchange diffusion» occurs (USSING 1947) — or better, »carrier exchange diffusion», as »exchange diffusion» has been used in an other sense (TEORELL 1947) — the formula is not correct. The same is true of the following treatment. There are, however, no proofs that such diffusion occurs in frog skin, and the results of this investigation seem to indicate that it must be quite unimportant. Carrier exchange diffusion has therefore been disregarded in the calculations.

Equation (1.2.1) can be transformed to

$$RT \ln (-\Phi_2^i / \Phi_1^i) = RT \ln (a_2^i \xi^{z^i} / a_1^i). \qquad (1.2.3)$$

By definition (cf. GUGGENHEIM 1950)

$$\mu^i = RT \ln a^i = z^i F \varphi + RT \ln (a^0 c^i f^i) \qquad (1.2.4)$$

where μ^i is the electrochemical potential, a^i the absolute (electrochemical) activity, φ the electrical potential c^i the concentration f^i the activity coefficient, and a^0 a constant for solutions in the same medium at constant temperature.

In the case of two electrolyte solutions, 1 and 2, of different composition in the same medium, (1.2.4) gives after substitution from eqn. (1.2.2)

$$\mu_2^i - \mu_1^i = RT \ln \frac{c_2^i f_2^i \xi^{z^i}}{c_1^i f_1^i} = RT \ln \frac{a_2^i \xi^{z^i}}{a_1^i}. \qquad (1.2.5)$$

Comparing (1.2.3) with (1.2.5), it will be seen that (1.2.3) expresses the difference in the total — in this case electrochemical — potential between two phases.

Equilibrium condition: In the special case when the net flux of the ions is zero, i.e. $\Phi_1^i = -\Phi_2^i$, equation (1.2.1) reduces to

$$\xi^{z^i} = a_1^i / a_2^i \text{ or } \varphi = (RT/F) \ln (a_1^i / a_2^i). \qquad (1.2.6)$$

This is an equilibrium condition for diffusible ions which was pointed out by TEORELL (1935 a) to be more generally valid than the similar Gibbs-Donnan case, which demands the presence of an *in*diffusible ion. It was verified experimentally for the case when the electrical potential was produced by a diffusing salt (TEORELL 1937). It is now also used to relate membrane resting potential of nerve and muscle at the equilibrium concentrations of diffusible ions (BOYLE and CONWAY 1941, HODGKIN 1951). In this case there is no true Donnan equilibrium due to an indiffusible ion but a dynamic equilibrium due to the presence of a virtually impermeable ion, Na^+, which is actively extruded from the cells.

b. ***Membranes in which active transport takes place.*** Independently of the character of the active transport mechanism which acts on an ion in the membrane, the result of its action on the actively transported ion B_a^i can be described as a deviation from the ratio $-\Phi_2^i / \Phi_1^i$, imposed by equation (1.2.1) (cf. USSING 1949 c). If the actively transporting force tends to move the ion from 1 to 2, the deviation from equation (1.2.1) may, in accordance with the principle of superposition of forces (cf. TEORELL 1949 a, 1952), be expressed by

$$-\Phi_{a2}^i / \Phi_{a1}^i = a_{a2}^i \xi^{z^i} / a_{a1}^i \xi_a^{z^i} . \qquad (1.2.7)$$

$RT \ln \xi_a^{z^i}$ defines the »active» potential in the membrane which acts on the actively transported ion B_a^i. This potential can be expressed in electrical terms as an electrical transport potential (cf. USSING and ZEHRAN 1951), according to the equation

$$\varphi_a^i = (RT / F) \ln \xi_a^i . \qquad (1.2.8)$$

It may also be defined in terms of the equilibrium state of the actively transported ion analogously to eqn. (1.2.6).

The active transport potential φ_a^i contributes to the *total potential* acting on the actively transported ion in the membrane, in which are thus included the chemical, electrical and active transport potentials. Other potential gradients are assumed not to exist in the system. The case where another potential gradient due to streaming of water enters into the system will be considered later. In the same way as eqn. (1.2.1) may be transformed to express the difference in electro-

chemical potential between an ion in solution 2 and in solution 1 in a system without active transport, eqn. (1.2.7) may be transformed to express the difference in the total or absolute potential (in conformity with the definition in 1.1) of the actively transported ion between solution 2 and 1. Thus

$$\mu^i_{a2} - \mu^i_{a1} = RT \ln \frac{-\Phi^i_{a2}}{\Phi^i_{a1}} = RT \ln \frac{a^i_{a2}\, \xi^{z^i}}{a^i_{a1}\, \xi^{z^i}_a} \qquad (1.2.9)$$

where μ'_a, the total potential, thus includes both chemical, electrical and »active» potentials.

Generally, it can be stated that if the fluxes of an ion in both directions are determined simultaneously, and if the activities of the ion on both sides of the membrane and the electrical potential difference across the membrane are known, it is possible to say whether the ion is actively transported (cf. Ussing 1951). The condition of no net flux of the solvent must be fulfilled, however, as well as that of a steady state.

1.3. Relation between the flux of an ion and its partial conductance.

There is another more indirect way of estimating whether active transport occurs or not. If the flux of an ion is determined in one direction and the partial conductance of the ion through the membrane calculated from this with due regard to electrochemical potential differences, and if it is found that the partial conductance of the ion (with the addition of the partial conductances of other (passive) ions, if known) exceeds the value of the total conductance as measured by electrical methods, it may be postulated that there is an active transport of the ion in the same direction as the flux was measured. Evidence of this kind has been used by Hogben (1951) to establish the active transport of Cl-ions through the gastric mucosa. If »carrier exchange diffusion» occurs this statement is not valid. A carrier exchange diffusion would not contribute to the conductance, but might

well contribute to the flux in either direction. In order to arrive at the connection between the flux of an ion and its partial conductance, the general expressions for the conductance of a membrane will first be discussed and then related to flux equations.

a. ***Definition of membrane conductance.*** Formulas for the conductance of a membrane between electrolyte solutions have been derived earlier by GOLDMAN (1943), HODGKIN and KATZ (1949), and TEORELL (1951). Various assumptions as to the character of the membrane and the distribution of the ions in the membrane have been made. Since the membrane often behaves as a non-linear resistance (COLE 1947, TEORELL 1948) the conductance G, of a membrane for a direct current is defined by (cf. TEORELL 1951, HODGKIN 1951)

$$G \quad -\partial I / \partial q' = -(F^2 / RT)\, \partial \Sigma \Phi^i / \partial \ln \xi^{z^i} \qquad (1.3.1)$$

where I is the current through unit area of the membrane in the direction from 1 to 2 in a system like that in 1.2 and Φ^i, the net flux of the ion, is defined by

$$\Phi^i = \Phi^i_1 + \Phi^i_2. \qquad (1.3.2)$$

In work with radioactive isotopes it is often of interest to estimate the partial conductance of an ion, i.e. the component of the membrane conductance due to current carried by ions of the species in question. Expressions for partial conductances under equilibrium conditions have been derived by HODGKIN (unpubl., quoted by KEYNES 1949) and HODGKIN and HUXLEY (1952 unpubl., quoted by HODGKIN 1951), and have also been used by USSING and ZERAHN (1951). Similarly to the total conductance, the partial conductance of an ion B^i is defined by

$$G^i = -(F^2 / RT)\, \partial \Phi^i / \partial \ln \xi^{z^i} \qquad (1.3.3)$$

and

$$G = \Sigma G^i. \qquad (1.3.4)$$

b. ***Expressions for the flux of an ion related to its partial conductance.*** In order to connect the partial conductance with the flux of an ion we introduce the general flux equation. The principles of the flux equation for an ion were formulated by NERNST (1888, 1889), and its application to diffusion of ions further discussed by PLANCK (1890), BEHN (1897), HENDERSON (1907, 1908), and others. It has been applied to diffusion through membranes by many workers, and recently TEORELL (1951) has extended its applicability to charged or ionic membranes.

In earlier treatments of problems concerning diffusion through membranes and their conductance, it has usually been assumed that they are homogeneous and that the ions diffuse through them as in free solution. Assumptions concerning the distribution of the ions in the membrane vary. HENDERSON assumes a »mixture boundary« (cf MACINNES 1947, p. 232). The »constant field assumption« (GOLDMAN 1943, HODGKIN and KATZ 1949) works with a linear electrical potential gradient ($d\varphi / dx = \text{const.}$). PLANCK's »constrained diffusion boundary« treatment seems to be generally preferable for treatment of membrane diffusion (cf. TEORELL 1951), though it makes the mathematical procedure more difficult.

In the case of a single electrolyte on either side of a non-charged homogeneous membrane, both PLANCK's and HENDERSON's assumptions give a linear concentration gradient through the membrane. In the case of electrolyte mixtures as well, a constant concentration gradient may be a good approximation as long as the mobilities of the different cations and anions do not differ too much.

The flux of an ion through a membrane in a system like that considered in 1.2, in which no active transport of ions occurs and in which the membrane is assumed to be homogenous, can be written in a compact form (TEORELL 1951) as

$$\Phi^i = - u^i c^i \, d\mu^i / dx. \qquad (1.3.5)$$

Here Φ^i is the net amount of an ion B^i which crosses a unit area of the membrane in the direction along the positive x-axis, which is perpendicular to the membrane, i.e. from 1

to 2, in unit time, u^i the absolute mobility, i.e. velocity per unit force, c^i the concentration of the ion in the membrane, and μ^i its electrochemical potential. x is the distance along the x-axis. At the boundary between solution 1 and the membrane, $x = 0$, and at the boundary between the membrane and solution 2, $x = \delta$.

After substituting from eqn. (1.2.2) and differentiating with respect to x, eqn. (1.2.4) gives

$$d\mu^i / dx = RT\, d \ln a^i / dx = RT\, d \ln (c^i f^i \xi^{z^i}) / dx \qquad (1.3.6)$$

and eqn. (1.3.5) may then be expressed as

$$\Phi^i = -u^i c^i RT\, d \ln (c^i f^i \xi^{z^i}) / dx \qquad (1.3.7)$$

which, except for the activity coefficient, f^i, is the formulation of the NERNST-PLANCK diffusion equation given by TEORELL (1951). The formula has been integrated between two boundaries when the activity coefficients have been neglected. As the variation of the activity coefficients is rather large in the concentration range encountered in some biological systems (being about 0.75 in a 0.1 M solution and 0.90 - 0.93 in 0.01 0.005 M solutions) its neglect may introduce a considerable error. However, it is also possible to integrate eqn. (1.3.7) when only 1-1 valent electrolytes like NaCl and KCl are considered. It is assumed that u^i does not vary with concentration, and this seems to be very nearly true in the actual concentration range as estimated from the transference numbers. For solutions of 1-1 valent electrolytes, the ionic strength is equal to the total concentration, c, of the salts, and by means of the DEBYE-HÜCKEL formula for the activity coefficient this can be expressed in terms of concentration. A simplified form of the DEBYE-HÜCKEL formula (GUGGENHEIM and SCHINDLER 1934) has been used:

$$-\ln f^i = 1.15 \sqrt{c} / (1 + \sqrt{c}). \qquad (1.3.8)$$

This is probably correct within a few per cent in the concentration range usual in biological systems for all the ions of common 1-1 valent electrolytes like NaCl and KCl.

If we consider an uncharged, homogeneous membrane in which ions diffuse as in free solution and assume that in the fraction of the membrane where diffusion occurs, α = »the effective diffusion area«, the concentration of the ions at the boundaries 1 and 2 is equal to that of the adjacent solutions, the integration of eqn. (1.3.7) between the two boundaries, 1 and 2, of the membrane where $x = 0$ and $x = \delta$ gives

$$\Phi^i = -\frac{\alpha\, R\, T}{\delta} \cdot \frac{u^i \{(c_2 - c_1) - \psi\} \ln \left(a_2\, \xi^{z^i} / a_1\right)}{\left(a_2\, \xi^{z^i} - a_1\right) \ln (a_2 / a_1)} \cdot \left(a_2^i\, \xi^{z^i} - a_1^i\right) \quad (1.3.9)$$

where c and a refer to the total concentration and the corresponding activity of the electrolytes and

$$\psi = 1.15 \left[\left(\sqrt{c_2} - \frac{1}{1+\sqrt{c_2}} \right) - \left(\sqrt{c_1} - \frac{1}{1+\sqrt{c_1}} \right) + 2 \ln \frac{1+\sqrt{c_1}}{1+\sqrt{c_2}} \right]. \quad (1.3.10)$$

Except for the correction for activities, eqn. (1.3.9) is of a form similar to that of the flux equations derived by BEHN (1897) and TEORELL (1951). The method proposed by TEORELL (1951) for integration of the differential flux formula has been used. It should be remarked that in the derivation used here the activity gradient through the membrane is linear, not the concentration gradient, as in the PLANCK case. The numerical value of ψ can easily be obtained from a graph when one of the concentrations is constant.

In the case of a single 1-1 valent electrolyte, the case we will be dealing with, we obtain from (1.3.9)

$$\Phi^i = -\frac{\alpha\, R\, T}{\delta} \cdot \frac{u^i \{(c_2 - c_1) - \psi\} \ln \left(a_2\, \xi^{z^i} / a_1\right)}{\ln (a_2 / a_1)}. \quad (1.3.11)$$

It may be asked what advantage this derivation of the flux formula has over that where activity coefficients are neglected. (The activity coefficients of the ions inside the membrane are uncertain, and if the membrane is assumed to be oil-phase, they may have quite other values than in water solution). It will be seen later that the factor within brackets in the

flux formula containing the concentration terms and ψ enters into the expression for the partial conductance of the ion, while the potential factor, which in this formulation contains only activities, is expressed in accordance with eqns. (1.2.3) and (1.3.7). From this latter point of view it is immaterial which function relates f to c, provided the activity coefficient, f, is the same for all ions.

The partial conductance may be obtained from the flux equation by differentiating with respect to φ, or more easily, with respect to $\ln \xi^{z^i}$ (cf. TEORELL 1951). Only in the case of a single electrolyte on both sides of the membrane is this expression simple. The rather complicated expression for the total conductance of a charged, ionic membrane with a single electrolyte on both sides was derived by TEORELL (1951). The partial conductances may be derived in a similar way.

Here, only the simple case of a system with one 1-1 valent electrolyte on both sides of the uncharged, »neutral», homogeneous membrane will be treated. From (1.3.11) we obtain directly

$$G^i = \frac{\alpha F^2}{\delta} \cdot \frac{u^i \{ (c_2 - c_1) - \psi \}}{\ln (a_2 / a_1)} = \underset{(c_1 = c_2)}{\frac{F^2}{\delta} \cdot u^i c}. \qquad (1.3.12)$$

Substituting for G^i in (1.3.11) we get

$$\Phi^i = -\frac{G^i}{F} \left\{ \frac{RT}{F} \ln \frac{a_2 \xi^{z^i}}{a_1} \right\} = \underset{(c_1 = c_2)}{-\frac{G^i}{F} \left\{ \frac{RT}{F} \ln \xi^{z^i} \right\}}. \qquad (1.3.13)$$

The term within brackets in (1.3.13) is the difference in electrochemical potential, expressed in terms of electrical potential between 2 and 1, and (1.3.13) thus states that the current borne by an ion is the product of its partial conductance and the potential difference between 2 and 1. This is an analogy to Ohms law, applied to an ion species, and is valid in the single salt case with the above mentioned restrictions as to the nature of the membrane. Combining eqn. (1.3.13) with (1.2.1) and 1.3.2), the following relationships between the partial conductance of an ion and its flux in either direction is obtained:

$$G^i = \Phi_1^i \frac{F\left(\frac{a_2\ \xi^{z^i}}{a_1} - 1\right)}{\frac{RT}{F} \ln \frac{a_2\ \xi^{z^i}}{a_1}} = \Phi_2^i \frac{F\left(\frac{a_1}{a_2\ \xi^{z^i}} - 1\right)}{\frac{RT}{F} \ln \frac{a_2\ \xi^{z^i}}{a_1}}. \qquad (1.3.14)$$

When the potential term $\ln (a_2\ \xi^{z^i} / a_1)$ is zero, i.e. at equilibrium for the ion in question, eqn. (1.3.14) reduces to

$$G^i = \Phi_1^i \cdot F^2 / RT = \Phi_2^i \cdot F^2 / RT \qquad (1.3.15)$$

This is the equation derived by HODGKIN (cf. KEYNES 1949) for the partial conductance at equilibrium, i.e. when $\Phi_1^i = -\Phi_2^i$. As all factors on the right hand side of the expression (1.3.14) can be estimated, the partial conductance of an ion of a single salt may thus be *experimentally* determined in a membrane in which no active transport occurs, when we know the flux of the ion in one direction, obtained with the aid of its isotope, the activities of the ion on either side of the membrane, and the electrical potential across it.

1.4. The flux and the partial conductance of an ion of a single salt through a membrane in which active transport occurs.

If one ion is actively transported through the membrane the formulas derived above are not valid. We will now consider a homogeneous membrane as in 1.3.b in which one ion of a single salt in the surrounding solutions is exposed to an active transport potential in the membrane, which tends to move the ion from 1 to 2. In accordance with the definition of the active transport potential in 1.2,b, the flux equation for an actively transported ion may be written

$$\Phi_a^i = -u_a^i c_a^i \, d\mu_a^i / dx - u_a^i c_a^i RT d \ln (c_a^i f_a^i \xi^{z^i} / \xi_a^{z^i}) / dx. \qquad (1.4.1)$$

Combining (1.4.1) with the corresponding eqn. for a passive ion (1.3.7) these equations can be integrated between the

boundaries 1 and 2 of the membrane, using a procedure similar to that employed for the integration of (1.3.7). The $\xi_a^{z^i}$ in eqn. (1.4.1) makes the treatment slightly more complicated. The active transport potential is assumed to be constant in the steady state and independent of the electrical potential difference across the membrane.

The integrated flux formulas for the active ion and for the passive ion contain, besides the correction factor for activities, a function of $\xi_a^{z^i}$. Both enter into the expressions for the partial conductances G_a^i and G^i, which are obtained when the flux formulas are differentiated with respect to $\ln \xi^{z^i}$. On substituting the partial conductance in the flux formula, we get

$$\Phi_a^i = -\frac{G_a^i}{F}\left\{\frac{RT}{F}\ln\frac{a_2\ \xi^{z^i}}{a_1\ \xi_a^{z^i}}\right\} = \underset{(c_1 = c_2)}{-\frac{G_a^i}{F}\left\{\frac{RT}{F}\ln\frac{\xi^{z^i}}{\xi_a^{z^i}}\right\}} \qquad (1.4.2)$$

which is the flux formula for the actively transported ion. The corresponding formula for the passive ion is obtained in the same form as (1.3.13). Differentiating these flux formulas with respect to $\ln \xi^{z^i}$ gives the partial conductances in the same form as in (1.3.3).

By combining eqn. (1.4.2) with (1.2.7) and (1.3.2), we get

$$G_a^i = \Phi_{a1}^i \frac{F\left(\dfrac{a_2\ \xi^{z^i}}{a_1\ \xi_a^{z^i}} - 1\right)}{\dfrac{RT}{F}\ln\dfrac{a_2\ \xi^{z^i}}{a_1\ \xi_a^{z^i}}} = \Phi_{a2}^i \frac{F\left(\dfrac{a_1\ \xi_a^{z^i}}{a_2\ \xi^{z^i}} - 1\right)}{\dfrac{RT}{F}\ln\dfrac{a_2\ \xi^{z^i}}{a_1\ \xi_a^{z^i}}}. \qquad (1.4.3)$$

When the potential term $\ln\left(a_2\ \xi^{z^i}/a_1\ \xi_a^{z^i}\right)$ is zero, (1.4.3) reduces to the simple from of (1.3.15).

It is evident from equation (1.4.3) that the partial conductance of an actively transported ion cannot be determined from measurements of the ionic flux in one direction, the electrical potential and the activities of the ion on both sides of the membrane, as in the case of a passive ion. It is also necessary

to determine the active transport potential. This as a rule also requires the simultaneous measurement of the fluxes in opposite directions of the actively transported ion. Other ways of determining the active transport potential will be considered later.

1.5. The active transport of a salt across a membrane.

If we consider the active transport of the salt across the membrane in a system like that in 1.2, which contains a single 1-1 valent electrolyte and in which the positive ion is influenced by a force in the membrane tending to transport it from 1 to 2, the following equation is obtained for the condition of electroneutrality when no electrical current is allowed to flow in an outer circuit.

$$\Phi_a^+ = \Phi^- = \Phi^{\text{salt}}. \tag{1.5.1}$$

The effect of H^+ and OH^- ions is neglected owing to their low concentration in solutions with pH values about 7. It is also assumed that no ions are formed in the membrane itself (in connection with the processes of active transport) which can diffuse out from it.

Although it is not of direct interest for the transport of salt through the membrane, the case when there is an electric current flowing through the system will also be considered, as the formulas derived in this connection have been used in the calculations of data from experiments with current flowing. The system is supposed to be »short-circuited», i.e. there is an outer circuit in which current can flow, connected to solution 1 and 2 by means of reversible electrodes. By means of an adjustable electromotive force in this outer circuit, the electrical potential across the membrane can be varied. The current I in the outer circuit is said to be positive in the direction 2 to 1. Then

$$\Phi_a^+ - \Phi^- = I / F. \tag{1.5.2}$$

By combining eqns. (1.5.1) or (1.5.2) with (1.4.2), (1.3.13), (1.2.2) and (1.2.8) and solving for φ and φ_a^+ the following equations are obtained:

$$\varphi = \frac{G_a^+ \varphi_a^+ - (G_a^+ - G^-)(RT/F) \ln (a_2/a_1) - I}{G_a^+ + G^-} \tag{1.5.3}$$

$$\varphi_a^+ = \frac{\varphi (G_a^+ + G^-) + (G_a^+ - G^-)(RT/F) \ln (a_2/a_1) + I}{G_a^+} \tag{1.5.4}$$

which are equally valid for $I = 0$. When $I = 0$ the net transport of salt

$$\Phi^{\text{salt}} = \Phi_a^+ = \Phi^- = \frac{G_a^+ G^- \{\varphi_a^+ - 2(RT/F) \ln (a_2/a_1)\}}{F(G_a^+ + G^-)}. \tag{1.5.5}$$

When $\xi_a^+ = 1$, i.e. $\varphi_a^+ = 0$, after substitution of the expressions for G from eqn. (1.3.12) eqn. (1.5.5.) reduces, if the correction for activity is disregarded, to the simple Nernst diffusion formula for a single salt, and eqn. (1.5.3) to the corresponding equation for the diffusion potential.

The equilibrium condition, i.e. when there is no net transfer of positive or negative ions, is defined by

$$\varphi_a^+ = 2(RT/F) \ln (a_2/a_1) \quad \text{or} \quad \sqrt{\xi_a^+} = \xi = a_2/a_1 . \tag{1.5.6}$$

1.6. Some considerations regarding the factors influencing the magnitude of the active transport of salt.

a. ***The connection between the experimentally determined active transport potential and the actual transport potential of the postulated „pump„ mechanism.*** It may by assumed that a biological membrane is not homogeneous and that the actively transported ions are exposed to the active force only in some parts of the membrane, while in other parts it behaves like a passive ion. In the case of frog skin we may think of small intercellular spaces, for instance. We consider

the same system as in 1.5 and indicate the quantities relating to the positive ion B^+ in the part of the membrane where active transport takes place by the index a and those relating to the positive ions in the parts of the membrane where they behave as passive ions by the index p. Then the actual transport potential of the pump mechanism,

$$\varphi_a^+ = (RT/F) \ln \xi_a^+ \tag{1.6.1}$$

by reasoning analogous to that used in connection with eqn. (1.2.7), is determined by

$$- \Phi_{a2}^+ / \Phi_{a1}^+ - a_{a2}^+ \xi / a_{a1}^+ \xi_a^+ . \tag{1.6.2}$$

With tracers it is impossible to determine this ratio, as it is not possible to decide which ions that have been actively transported and which passively. If the »pump» is looked upon as an electrical battery short-circuited by a conductor with a conductance corresponding to that of the passive positive ions, it is easy to show that φ_a^+ is related to φ_a^+, the experimentally determined active transport potential, by

$$\varphi_a^+ = \varphi_a^+ \cdot G_a^+ / (G_a^+ + G_p^+) . \tag{1.6.3}$$

The »internal» efficiency of the pump mechanism is not considered here, but the factor $G_a^+ / (G_a^+ + G_p^+)$ may be said to be an efficiency factor describing the external working conditions of the pump, i.e. it expresses how much of the total pump potential is used for the active transport of salt. Another way of putting the same thing is to say that it expresses how much of the pump potential remains when the »shorting» effect of passive positive ions has been subtracted.

It seems rather probable that in several cases where an increased permeability of the biological membrane occurs, this is essentially due to an increased conductance for passive ions. If φ_a^+ and G_a^+ are unchanged while G_p^+ increases, this results in a decreased φ_a^+ as determined experimentally.

b. ***Effects of changes in conductance on the active transport of salt.*** We are still considering the system of 1.5. For a membrane with a constant total conductance $G = G_a^+ + G^-$, assuming φ_a^+ and the chemical potential difference to be constant, it is evident from (1.5.5) that the maximum net transport of salt, Φ^{salt}, occurs when the product of G_a^+ and G^- is a maximum, i.e. when $G_a^+ = G^-$.

In the case of a membrane in which only G^- or G_a^+ changes while φ_a^+ and the chemical potential are constant, $|\Phi^{\text{salt}}|$ changes in the same direction as the conductance, as is evident from an inspection of the derivatives $\partial \Phi^{\text{salt}} / \partial G_a^+$ and $\partial \Phi^{\text{salt}} / \partial G^-$. The direction of Φ^{salt} is determined by the potential term $\{\varphi_a^+ - 2(RT/F) \ln (a_2/a_1)\}$. If this is positive, the larger it is the more salt is transported in the direction of the active transport potential, and if it is negative, the larger it is the more the passive diffusion due to concentration differences between 1 and 2 overcomes the active transport.

In this connection the reasoning above concerning the relation between φ_a^i and φ_a^+ is of importance. Assuming φ_a^- and G_a^+ to be approximately unchanged while G_p^+ varies, different values of the effective transport potential φ_a^- are obtained. The higher G_p^+ the lower the effective potential and the smaller the Φ^{salt}.

1.7. The possibilities of estimating the active transport potential, φ_a^i, when the flux of an ion is measured only in one direction.

There are often technical difficulties which make it inconvenient to measure the flux in the two opposite directions simultaneously. There are, however, other ways of estimating the active transport potential φ_a^i than by means of (1.2.7). In the simple case considered in 1.5 with two solutions of the same 1-1 valent electrolyte on both sides of the membrane, it is possible to use eqn. (1.5.4) to obtain φ_a^+ if the partial conductances of the two ions are known, as well as their activities and the electrical potential across the membrane. The

partial conductance of the passive negative ion may be determined from measurement of its flux in one direction, using for instance its radioactive isotope, by substituting this value in eqn. (1.3.14). If the total conductance G of the membrane is then determined by means of electrical conductance measurements, the partial conductance of the positive ion is obtained from (1.3.4). Substitution of the values of G_a^+ and G in eqn. (1.5.4) gives φ_a^+. In the case when $\varphi = 0$ and $a_1 = a_2$, eqn. (1.5.4) reduces to (cf. USSING and ZERAHN (1951))

$$\varphi_a = I / G_a^+ . \tag{1.7.1}$$

The φ_a^+ obtained by this method will in the following be referred to as $\varphi_{a\mathrm{I}}^+$. (»Method I.»)

It is also possible to obtain φ_a^+ from measurements of the fluxes of the two ions at the same time in the same direction. In this case also, the experiment may be made with or without a current flowing in an outer circuit. As the case with current flowing is the most general one and has been used in the experiments, the formulas derived will be given for this case. By combining (1.5.2) with (1.2.1), (1.2.2), (1.2.7), (1.2.8) and (1.3.2) and solving for φ_a^+ the following equation is obtained:

$$\varphi_a^+ = \varphi + \frac{RT}{F} \ln \frac{a_2}{a_1} - \frac{RT}{F} \ln \left\{ 1 - \frac{\Phi_1}{\Phi_{a1}^+} \left(1 - \frac{a_2}{a_1 \xi} \right) - \frac{I}{F \cdot \Phi_{a1}^+} \right\} =$$

$$= \varphi + \frac{RT}{F} \ln \frac{a_2}{a_1} + \frac{RT}{F} \ln \left\{ 1 - \frac{\Phi_2^+}{\Phi_{a2}^+} \left(1 - \frac{a_1 \xi}{a_2} \right) - \frac{I}{F \cdot \Phi_{a2}^+} \right\}. \tag{1.7.2}$$

When $a_1 = a_2$ and $\xi = 1$, i.e. $\varphi = 0$, the expression reduces to

$$\varphi_a^+ = \frac{RT}{F} \ln \left\{ \frac{\Phi_{a1}^+}{\Phi_{a1}^+ - I/F} \right\} = \frac{RT}{F} \ln \left\{ \frac{\Phi_{a2}^+ - I/F}{\Phi_{a2}^+} \right\} \tag{1.7.3}$$

which is the form used by USSING and ZEHRAN (1951) to calculate the sodium transport potential of frog skin. The φ_a^+ obtained by the latter method, i.e. from eqns. (1.7.2) and (1.7.3), will from now on be referred to as $\varphi_{a\mathrm{II}}^+$. (»Method II».)

1.8. Water movement through the membrane.

If the membrane is permeable to water there may be a net flux or streaming of water through the membrane when the activity of the water in 1 and 2 differs. Then by analogy with eqn. (1.2.1), we have

$$- \Phi^w_2 / \Phi^w_1 = a^w_2 / a^w_1 \tag{1.8.1}$$

or, if the friction between water molecules in the diffusion process of water molecules into water is taken into consideration, the formula given by USSING (1949 c) (which may be more correct, though experimental verification is lacking) is obtained:

$$- \Phi^w_2 / \Phi^w_1 = (a^w_2 / a^w_1)^2 . \tag{1.8.2}$$

These formulas do not take into account the possible influence of electro-endosmotic phenomena which may be of importance in charged membranes (SCHMID 1950).

If we now assume a force inside the membrane which tends to transport water molecules from 1 to 2 we may write, analogously to eqn. (1.2.7),

$$\frac{- \Phi^w_{a2}}{\Phi^w_{a1}} = \frac{\Phi^w_{a1} - \Phi^w_a}{\Phi^w_{a1}} = \frac{\Phi^w_{a2}}{\Phi^w_{a2} - \Phi^w_a} = \left(\frac{a^w_{a2}}{a^w_{a1}\, \xi^w_a} \right)^2 \tag{1.8.3}$$

from which ξ^w_a can be obtained when the net flux, the flux in one direction, and the activities of the water are known.

The formulas given do not allow an estimation of the absolute rate of water movement in the membrane unless the structure and thickness of the membrane is determined. This, however, is usually impossible in the case of biological membranes.

Just as $\ln \xi^{z^i}_a$ may be considered as a mean active transport potential to which the ion B^i is subjected in an inhomogenous biological membrane, $\ln \xi^w_a$ is the mean transport potential to which the water molecules are subjected. This should be related to the true transport potential of a water pump mechanism by an expression similar to (1.6.3). It may thus be assumed

that the active transport potential to which the water molecules are subjected in the part of the membrane where active transport of water takes place, $\ln \xi^w_a$, is greater than this mean $\ln \xi^w_{\prime\prime}$.

1.9. The effect of water movement on the diffusion of ions.

The transfer of water (solvent) through a membrane is of interest for its own sake, but it is also of importance because it affects the flux of solutes (ions). The equations below were derived in order to make possible a rough quantitative estimation of the effect of a streaming of water through the frog skin on the flux of ions. The problem has been considered by USSING (1949 c) in connection with experimental work on frog skin. Although it may be doubtful if some of the assumptions made by USSING in his derivation are valid for frog skin, it seems, at present at least, to be the best approach to a quantitative treatment of these phenomena. In his formula (14), the friction coefficients between ion and water and between water and water molecules are used in an expression for the force with which the water movement due to osmosis affects the ions. If the inverted values of the diffusion coefficients of ion (D^i) and water (D^w) are used instead of friction coefficients, USSING's formula, written with the present notation, assumes the form

$$- \Phi^i_2 \,/\, \Phi^i_1 = a^i_2 \, \xi^{z^i} \,/\, a^i_1 \, \xi^i_{iw} \tag{1.9.1}$$

where

$$\xi^i_{iw} = (a^w_1 \,/\, a^w_2)^{D^w/D^i} = (\Phi^w_1 \,/\, - \Phi^w_2)^{D^w/2D^i} . \tag{1.9.2}$$

A relevant treatment which involves the resultant rate of flow has been discussed by TEORELL (1951), who has also, on the same basis, derived a formula valid for charged membranes, of a form which is related to (1.9.1) (TEORELL 1952[1]).

[1] Professor T. Teorell has kindly allowed me to quote from a manuscript in the press.

When there is active transport of water,

$$\xi^i_{iw} = (a^w_{a1}\, \xi^w_a / a^w_{a2})^{D^w/D^i} = (\Phi^w_{a1} / -\Phi^w_{a2})^{D^w/2D^i} \qquad (1.9.3)$$

from which the magnitude of ξ^i_{iw} may be estimated when eqn. (1.8.3) can be expressed in experimental quantities.

For an actively transported ion

$$-\Phi^i_{a2} / \Phi^i_{a1} = a^i_{a2}\, \xi^{z^i} / a^i_{a1}\, \xi^{z^i}_a\, \xi^i_{iw}. \qquad (1.9.4)$$

It may be that water is actively transported through the membrane in those sections where the active transport of ions takes place, and diffuses passively in other sections. In that case it seems probable that the greater part of the action on ions of water movement caused by active transport of water is included in $\ln \xi^{z'}_a$. Owing to the probable inhomogeneity of biological membranes, there is always a great deal of uncertainty as to the assumptions which are valid in a special case. However, until more experimental data have been assembled, it is probably best to assume the membrane to be homogeneous, and to explain deviations from theory as depending on inhomogeneities of the membranes, among other things.

Chapter 2.

The active transport of sodium ions through isolated frog skin.

2.1. Earlier experimental evidence for an active transport of ions through frog skin.

a. *Electrical phenomena connected with the active transport.* Du Bois-Reymond (1848) first recognized that frog skin can produce electrical currents and is the seat of an electromotive force. After him, several experimenters studied the electrical properties of frog skin. Their results are not conclusive, however, as the solutions they used on either side of the skin were not well defined.

Galeotti (1904) used chemically similar solutions on each side, and tested the effects of different salts on the potential difference across the skin. He showed that with sodium and lithium salts the isolated living skin maintained a considerable potential, the inside (chorion side) being positive as compared to the outside (epithelial side). With other salts the potential was low or zero. Dead skins always showed zero potential with the same salt solution on both sides. He concluded that the skin was more permeable to sodium and lithium from outside to inside than in the reserve direction, while K and Cl ions were equally permeable in both directions. His experiments are in fact fairly conclusive proof of an active transport of sodium (and lithium) through frog skin, or, at least, they show that with the same solution of sodium and lithium salts on both sides, some ion — from within the skin or from the bathing solutions — is transported preferentially in one direction. This requires expenditure of energy in the skin, as it can be stated generally that with the same solution on both sides of a membrane, i. e. a solution with the same chemical composition, at the same temperature and the same pressure, some process inside the membrane is necessary to

produce and maintain an electrical potential. (It might be that it is merely a leakage of ions out from the skin which produces the potential, but it seems improbable that in GALEOTTI's case this should occur only in lithium and sodium salt solutions, nor could it account for the long duration of the potential).

Several later investigators (ORBELI 1910, UHLENBROCK 1924, HASHIDA 1922, MOTOKAWA 1935, 1938 and others) thoroughly investigated the effect of different ions in different concentrations on the potential of frog skin. The literature is reviewed by GREVEN (1941). The potential was explained in physico-chemical terms of diffusion potentials, adsorption potentials or colloid chemical phenomena, though MOTOKAWA (1938) states that chemical reactions must be assumed to explain the (resting) potential.

Others have been interested in the relation between the metabolism or oxygen consumption of frog skin and its electrical potential or its ability to produce an electrical current. The earlier literature is reviewed by DEAN and GATTY (1937), and LUND and coworkers (1947) give a very full list of the pertinent literature. All this work shows that there is a close connection between the respiration and the potential, in so far as a reduced oxygen consumption is followed by a decrease in the potential or the current produced by the skin. We will return to this in a later chapter.

FRANCIS (1933) first studied the relation of the electrical energy produced to the total available energy as determined from measurements of the oxygen consumption in frog skin. If a frog skin, 1.6 cm^2 in area, was mounted with Ringer's solution on both sides and the circuit completed so that the total resistance was 1500 ohms, a current of 20–30 μA for 12 hours could be obtained. FRANCIS found the electrical energy output of the skin to be 5—10 per cent of the energy released by oxidation processes. Similar measurements have been performed by LUND and STAPP (1947) and HOLT (1947) who found the output of electrical energy from isolated frog skin, »shorted» by a low-resistance outer circuit to be 1—2 per cent of the total energy output from metabolic processes. These latter authors, however, disregard the energy produced by the electrical current, or rather, the ionic current inside the skin, and

since the skin has a high resistance to direct current their figures may be regarded as low. In the light of later investigations with isotopes, the experiments of FRANCIS and LUND and coworkers indicate quite a high efficiency of the skin in converting chemical into electrical energy, or more correctly, into the work of transporting ions.

b. *Observations on transfer of substances through frog skin.* As early as 1892, RIED established the ability of frog skin to transport substances from one side to the other. He found that fluid passed from the outside to the inside of the skin when it was bathed with normal saline on both sides. ADOLPH (1927), in his study of the adaptation of frogs to salt solutions of different concentrations, concluded that »other forces» than the osmotic pressure act at the skin, but HUF (1935, 1936 a) was the first to demonstrate that a transport of chloride takes place from the outside to the inside in isolated frog skin bathed with Ringer's solution on both sides. At the same time, there was a transport of water in the same direction. HUF believed that there is also an active transport of water, but his experiments do not exclude the possibility that this results from changes in the osmotic pressure produced by the salt transport (KROGH 1939). The transfer of salt and fluid, and the potential, could be inhibited by cyanide and monobromoacetate. The active transport and the potential of the monobromoacetate-poisoned skin could be restored with lactate or pyruvate but not with glucose or glucosediphosphate. In later work (HUF, PARRISH and WEATHERFORD 1951, HUF and PARISH 1951, HUF and WILLS 1951), factors influencing the active transport through frog skin have been further investigated.

KROGH (1937, 1939) made a study of the uptake of salt by the skin of living frogs under varying conditions. However, it was not until (radioactive) isotopes were used that more conclusive evidence could be obtained about the mechanism of the active transport. KATZIN (1939, 1940) used radioactive isotopes of potassium, sodium and rubidium in permeability experiments on frog skin. He was able to demonstrate that sodium passes through the skin at a greater rate inward than outward when the same mixture of sodium and potassium chloride was used

on both sides. His short communication does not make it clear whether he measured the potential difference across the skin, but as the inside of the skin is positive relative to the outside under the conditions of his experiments, his results may be taken to indicate an active transport of sodium.

Evidence that isolated frog skin can transport sodium, not only against an electrical potential gradient, but also against a steep concentration gradient, was given by USSING (1948, 1949). Some theoretically important considerations on the possibility of distinguishing between active transport and diffusion are made by USSING (1949 c). USSING and coworkers have also studied the influence of different factors on the transport of sodium through the skin, but we will return to that later. A recent review of this work is given by USSING (1951). An important finding is that all the electrical current which can be drawn from the skin seems usually to come from the active sodium transport (USSING and ZEHRAN 1951). In some cases, however, there is also evidence for an active transport of chloride outward through the skin.

The rest of this chapter is an account of some experiments in which the active transport through frog skin was studied by means of radioactive isotopes of sodium and chlorine combined with measurements of the electrical potential and resistance of the skin.

2.2. Methods.

a. ***Animals.*** The experiments were carried out on the isolated abdominal skin of the green frog, *Rana temporaria*. The frogs were collected during the autumn and kept in tanks of tap water in a cool cellar. The water was frequently renewed. The animals were not fed. Moulting of the animals was not controlled and some of the rather great variations in the properties of the skins may be due to this (BARKER JÖRGENSEN 1949). After pithing, the abdominal skin was removed by careful dissection in order not to stretch it.

b. ***Apparatus used for mounting of the skin.*** An apparatus similar to that described by USSING (1949 b) was used. The skins were mounted between two perspex half cells, shown in Figs. 1 and 2. The inner diameter of the cells was 3 cm, corresponding to a skin area of 7.1 cm^2

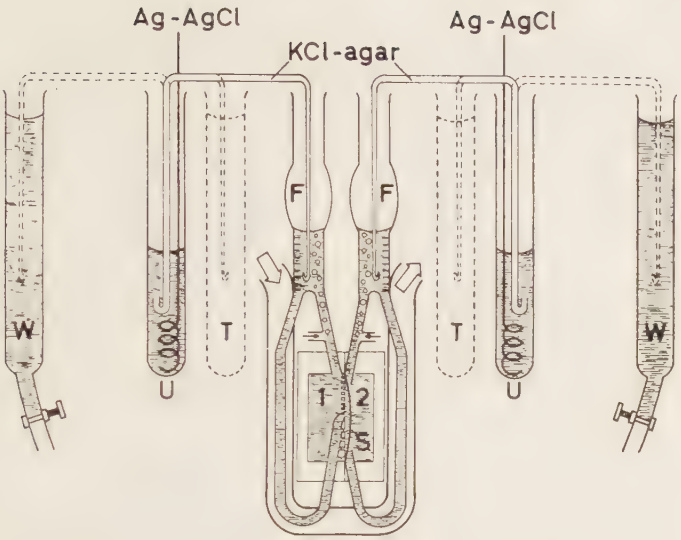

Fig. 1. Apparatus used for mounting frog skin. The skin (S) was mounted between two perspex half cells, 1 and 2. Gas, usually air, entered at the small arrows → and circulated the fluid in the two compartments. The big arrows ⇨ indicate constant temperature water which passed through a rubber tube surrounding the narrow tubes for the fluid circulating in the two compartments of the cell. The electrode and KCl bridge arrangement for potential measurements is also shown. The electrodes for resistance measurements are shown in Fig. 2. For further details see text.

between the compartments of the two half cells. The two ends of the half cells contained platinized platinum electrodes, E_{rp}, for the measurement of the conductance of the skin, and two smaller annular, similar electrodes, E_i, by which current could be forced through the skin from a »potential-controlling circuit». The half cell used on the outside of the skin, i.e. the epithelial side, in the following referred to as 1, had a thin fine-meshed sieve of perspex covering the opening. The skin could be pressed against this for volume measurements of the two compartments. There was a spiral of 0.2 mm platinum wire on the skin side of the sieve. One end of it passed out through the perspex wall. The wire was platinized and used as a recording electrode, E_a, in the resistance bridge arrangement. 8 silver nails around the periphery fixed the skin, and made mounting easier. They fitted into small holes in the opposite half cell.

In the middle of the sieve was the main inflow channel for the circulating fluid in compartment 1; the fluid exit was immediately on the other side of the sieve in the upper part of the cell. There were small inflow holes towards the skin side along the whole of the inflow channel, espe-

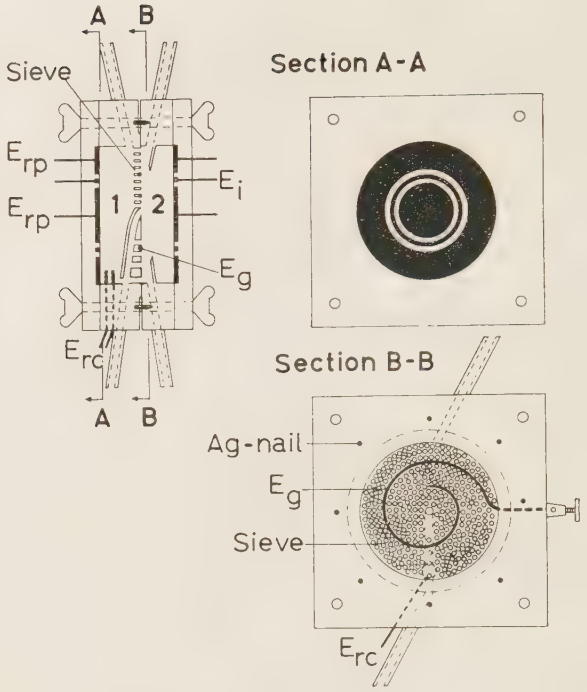

Fig. 2. The frog skin chamber. For details see text.

cially in the lower part of the cell. There was also a small inflow hole on the other side of the sieve at the lowest point of the cell, so that the chamber might be emptied completely. This hole was placed so that the flow from it passed between the two platinum electrodes, $E_{r.c.}$ used for measuring the conductance of the solution in 1. This also ensured good stirring in the lower part of the chamber.

In the other half cell, for the chorion side of the skin, the inlet and outlet tubes, which entered the chamber near the skin at the bottom and top respectively, protruded a little into the chamber. The end of the tubes were cut skew where they protruded into the chamber, and the hole opened out to enable the chamber to be drained and filled completely. With this arrangement, the potential across the skin, measured via the circulation channels, corresponded to the potential of a point in the solutions very near to the skin (cf. below 2.2.d).

The perspex tubes of the two half cells were connected to the rest of the circulation apparatus by means of rubber tubes. (Fig. 1). The apparatus consisted of two glass funnels, (F), one for each half cell, with a wider part to avoid foaming. There were two connections at the base of each funnel, one to the T-tube for the entrance of the gas which bubbled through

the solution and kept it circulating, and the other to a narrow (2 mm internal diameter) glass tube for connection with the inflow channel at the lowest part of the chamber. These glass tubes, one for each half cell, passed coaxially through a wide rubber tube, in which constant-temperature water was circulating. This arrangement made it possible to maintain the solutions in the half cells within a temperature range of $20°.0 \pm 0°.1$ C. This was essential for the conductance measurements. In this way, the error due to the changes in temperature were kept far below 1 per cent.

The gas — usually air — used for aerating and circulating the fluids, was saturated with water by passing it through a bubbling flask. In this way the loss of water by evaporation was kept at a minimum. There was some foaming especially after the addition of some drugs used in the experiments, and this may have increased the water loss a little.

c. ***Estimation of the volume of the circulating fluids.*** The two glass funnels, from which samples of the solutions could be drawn, were marked so that the height of the fluid meniscus could be read. It was found that the volume of the system below the funnels was constant when the apparatus was mounted in the same way from day to day. The volume was frequently controlled by filling the two sides with known volumes. The marks on the funnels allowed an estimation of a volume change of about 0.05 ml but the difficulty of getting the frog skin and the meniscus in the T-tube where gas was bubbled into the system always in the same positions made the readings more uncertain. They were probably correct within ± 0.1 ml. If the chamber was filled with a certain volume of solution, it could be sucked out and recovered to within 0.2 ml. The volume usually used on each side of the skin was 8 ml.

d. ***Measurement of the electrical potential, φ, across the skin.*** The electrical potential of the solutions on each side of the skin was led off from the funnels (F) by means of two saturated KCl-agar bridges which, with a very narrow opening directed upwards, dipped into the solution of the funnels (Fig. 1). The arrangement of the tubes connecting the funnels with the frog skin cell (2.2.b) was such that the potential in the funnels corresponded to the potential of a point in the solution near to the skin. This was of importance when there was a potential gradient through the solution in the cell (cf. 1.2.g). For the significance of the resistance in the measuring circuit, see below.

The KCl-bridges, when not in use, were placed in saturated KCl solution in the test tubes (T) and before use they were thoroughly washed in distilled water in the washing tube (W). It was difficult to obtain a bridge with so narrow an opening that the diffusion of KCl into the bathing solution could be neglected. This was important when measurements were made with dilute solutions in 1. By measuring the conductance of the solution as described below it was easy to check the KCl-bridges in this respect. It was found that the bridge used in 1 leaked into a very dilute salt solu-

tion about 0.06 — 0.12 μmol hr^{-1} when the bridge dipped into the solution for several hours. Such long periods often occurred when the same Ringer's solution was used on both sides of the skin. It may then be concluded that there was no appreciable change in the KCl concentration due to leakage from the bridge, the total change with 8 ml solution in 1 corresponding to about 0.1 mM KCl for a 6 hour period.

The leakage was a little greater when the bridge stayed in the solution for only a short period, amounting to about 30 — 70 $\mu\mu$mol sec^{-1}. When a dilute solution was used in 1 the bridge as a rule dipped into the solution for only about a minute for each potential measurement. As the measurements were made only once every 10 to 20 minutes, this means that the leakage of KCl from the bridge corresponded to a mean negative flux of salt, $-\Phi^{NaCl}$, of about 1 $\mu\mu$mol cm^{-2} sec^{-1} through the frog skin, and probably less (cf. below 2.2.f. and 2.3.C). The leakage from the bridge can therefore be neglected.

Another difficulty was to find bridges which behaved symmetrically with respect to each others when they were placed for several hours in Ringer's solution. This was often done when a current was forced through the skin to keep the potential difference equal to zero (see below). It was also necessary that several washings of the bridges in distilled water should not influence the potential measurements. Several bridges were tested in these respects, and some good pairs found.

The bridges were frequently compared with each other by making measurements in the same solution or in solutions of different concentrations. The washing tubes (W), connected by a saturated KCl bridge, were used in these control measurements. The accuracy with which the bridges recorded a potential was also checked by comparing them with bridges with wide openings, in which a liquid junction potential would be less likely to develop. As a result, it can be stated with confidence that the narrow-opening bridges introduced errors less than 1 mV even when measurements were made in dilute solutions, as compared with wide-opening bridges.

The other end of the KCl-bridges dipped into test tubes (U) containing saturated KCl solution with Ag-AgCl electrodes. The Ag-AgCl electrodes were frequently chloridized and always tested at the beginning and end of an experiment, at the same time as the bridges were tested, by connecting the tubes (U) with a KCl-bridge. They were found to keep constant for a considerable time.

The millivoltmeter used incorporated a direct-coupled amplifier of two push-pull stages. Two 6AK5 tubes were used for the input stage and the grids were connected directly to the electrodes without a grid leak. With proper voltages these tubes give a low grid current (cf. Parsegian 1946 and Nastuk and Hodgkin 1950). The plates were supplied with 40 volts and the cathodes were connected to a negative voltage of — 6 volt by a variable common cathode resistance of about 30 kΩ. The floating grid voltage was adjusted to the potential of the electrodes and the grid current was

frequently tested. It was less than 10^{-10} A. This means that the resistance in the measuring circuit which was of the order of 0.1 — 1 MΩ when dilute solutions were used in 1, affected the measurements by less than 1 per cent.

During the potential measurements, the system was earthed via the Erp-electrode in solution 2, while other electrodes could be disconnected. Streaming of the bathing fluids was found to change the potential readings only when dilute solutions were used in 1 (below 1—2 mN NaCl). For these solutions, gas bubbling was interrupted when the potential was measured.

The voltmeter was linear within 0 — 100 mV. A wider measuring range was seldom used. Its calibration was frequently checked with a millivolt-calibrator built up essentially of Muirhead resistance components and a standard potential cell. Its accuracy was estimated to be ± 1 per cent. In the millivoltmeter there was also a built-in fixed voltage standard of about 50 mV which was compared with the millivolt-calibrator mentioned. It was switched in between the two grids after each measurement. One of the grids was then earthed via the Ag-AgCl electrodes, the KCl-bridge, the solution in 1 and the E_{rp} electrode just as during the measurements of the potential across the frog skin. This built-in calibration was a check which was found to reduce possible errors to a minimum. Owing to the high cathode resistance the arrangement was very insensitive to variation in the electrode potentials with respect to earth. A change of this voltage of up to 0.5 V changed the scale but little, and such a change was easily detected by the use of the built-in voltage calibration. Readings of potential differences were made to the nearest mV.

The experimentally measured potential difference is the same as that defined in 1.2, if it is assumed, as is usually done (Bjerrum 1905), that there is a negligible liquid junction potential between the solution in which the potential is to be measured and the saturated KCl solution.

e. ***Resistance (conductance) measurements on frog skin.*** The d. c. resistance of the frog skin was measured with a bridge as indicated in Fig. 3. Square pulses of appropriate length were obtained from a square wave generator (SWG) working symmetrical to earth. They passed two 100 kΩ resistances (r_1) which formed two symmetrical bridge arms.

As the resistance of the object was small (usually only 0.1 to 1 per cent) compared with these resistances, practically constant current pulses were obtained throught the object. Another advantage with the high bridge circuit resistance was that the current drawn from the skin when connected to the bridge may be neglected. The third and fourth arms of the bridge were a precision decade resistance and the frog skin cell. A trimming condenser was connected parallel to the decade resistance. Between the cell and the decade resistance the bridge was connected to earth. All measurements were performed in a double Faraday cage.

The detector was a highly symmetrical direct-coupled push-pull amplifier connected to a cathode-ray tube. The input stage consisted of two EF50 tubes with a high cathode resistance and no grid leaks. The floating grid potential was adjusted near to earth potential. The voltage drop

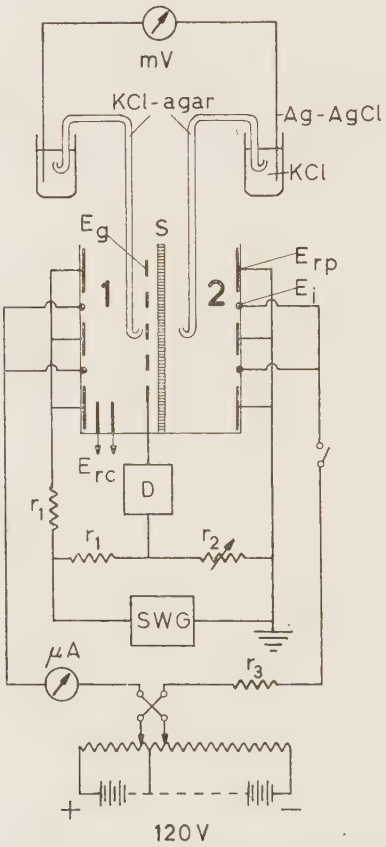

Fig. 3. Circuit of the bridge for measurements of the d.c. resistance of the frog skin. The arrangement for measurement of the electrical potential across the skin and the potential-controlling circuit are also shown. SWG is a low-frequency square wave generator. $r_1 = 100$ kΩ, r_2 = variable precision decade resistance. $r_3 = 200$ kΩ, is in series with the variable E.M.F. and the μA-meter in the potential controlling circuit. D indicates the detector, i.e. a d.c. amplifier and a cathode ray oscillograph. S indicates the frog skin. The circuit shows the three electrode system (cf. text). When measuring the conductance of the solution in 1, the E_{rc} electrodes could be connected to a similar bridge connected with a 1000 c.p.s. sine wave generator.

between the recording electrodes of the frog skin cell was applied to the grid of one of the input valves. The grid could be connected either to the E_{rp} electrode in 1 (2 electrode system) or to the E_g electrode (3 electrode system). The latter alternative was used in the measurements. This voltage drop could be compensated by injecting a voltage of the same amplitude, obtained across the precision resistance, into the grid of the second input valve.

When E_1 was connected to the grid and used as a recording electrode, polarization of the E_{rp} electrode in 1 introduced a negligible error. This was of importance, especially with dilute solutions in 1. An advantage was also that the potential drop through the badly conducting solution 1 was not recorded and higher accuracy could be obtained (cf. below). The resistance of the solution 1 was instead added to the resistance of one of the symmetrical bridge arms and could be corrected for, but as it caused an asymmetry of the bridge of only about 1 per cent even with a 1 mN sodium chloride solution in 1, and less than 0.1 per cent with Ringer's, the correction was usually omitted. Measurements with the 2 and 3 electrode system gave consistent d.c. resistance values of the skin.

The polarizability of the electrodes was checked by filling the cell with Ringer's solution (or dilutions of Ringer). Electrode polarization introduces no error at the current strength, amplifier sensitivity and time intervals used in this investigation.

Some remarks will be made on the *possibility of measuring the d.c. resistance of frog skin.* The method used here is essentially the same as that used by WEIDMANN (1949) to measure the »incremental d.c. resistance» of *Nitella* cell membrane. Frog skin behaves as a non-linear resistance element (GERSTNER 1939 b). For current densities of $\pm$ 20 μA cm^{-2} GERSTNER (1939 a) finds a linear current-voltage relation. However, his experiments were performed on skins with very low resting potential. His results do not agree with those in this investigation.

On the contrary, it should be stressed that it was usually found to be incorrect to calculate the d.c. resistance of the skin from the current necessary to reduce φ from say 50 mV to zero — which usually corresponded to about 20 μA cm^{-2}. The nonlinearity effects are a disturbing factor, and the error in the d.c. resistance value is of the order of 10—20 per cent or even more in some skins.

In the present series of experiments the density of the square current pulses was usually about 3 μA cm^{-2} of skin when measurements were made on low resistance skins, and lower for skins with high resistances. The voltage drop across the skin did not exceed 10 mV. If the current density was increased to double this value there was no change in the resistance values measured. Non-linearity effects can thus be excluded from the resistance measurements. With stronger currents, especially at the lowest frequencies, electrode polarization interfered with the measurements.

The behaviour of frog skin towards electrical currents can be represented by an electrical equivalent circuit, which contains a capacitive component, fairly large, in parallel with a resistance. Though the membrane capacity was not determined in the experiments reported in this paper, earlier unpublished measurements with the GRÜTZMACHER bridge method (GRÜTZMACHER 1934) and with transient analyses of square pulses (TEORELL 1946) have shown that capacities of 4—5 μF cm^{-2} and even more are usual figures with »normal» skins, and with skins exposed to the action of the diuretics used in the present experiments.

It is of the same order of magnitude as, or a little greater than the capacities of many cell membranes (COLE and CURTIS 1950, WEIDMAN 1951). It implies that there are not many active cell membranes coupled in series through the frog skin: possibly there is only one membrane of high resistance and large capacity. The membrane parallel resistance is usually about 1—2 kΩ cm^{-2} with Ringer's on both sides of the skin.

The big capacitive element of the skin gives it a long time constant, especially when the resistance is high, as when dilute solutions are used on the outside (membrane resistance of 10—20 kΩ cm^{-2} can occur). A current increment must then be of sufficient duration to allow the voltage to approach a new constant value, which means that square pulses of sufficiently low frequency are required.[1]

The voltage increment recorded with the arrangement described depends not only on the resistance of the skin but also on the resistance of the electrodes and the bathing fluids. The latter resistances may by determined by measuring the resistance of the cell without the skin and may then be subtracted from the total resistance of the cell with the skin to give the skin d.c. resistance. This method is, however, difficult to use when solutions of different concentration are used in 1 and 2. An other method of approach is to measure the high frequency impedance of the skin. Owing to the comparatively large capacity of the skin its high frequency impedance is small and can be neglected, as it is usually less than 1 per cent of the total

[1] The method of measuring the d.c. electrical resistance in a thin biological membrane with a big reactive element, probably an essentially dielectric capacity, differs somewhat from methods used for membranes with no or unimportant capacity, such as thick inanimate membranes. In such a case it is an advantage to use a relatively high frequency a.c., as with low frequencies, especially at high current densities, phenomena of so called »time dependent rectification» occur (TEORELL 1948, 1951, cf. also COLE 1947) due to a change in the distribution of ions in the membrane. Similar phenomena, also called »delayed rectification» (HODGKIN 1949), have been observed at low current densities in excitable biological membranes, for instance in nerves (COLE 1941) and *Nitella* (WEIDMANN 1950). They are such that it seems probable that a change takes place in the permeability of the membrane (HODGKIN 1949), and are probably related to the behaviour of the membrane which is the basis of the electrical activity phenomena (HODGKIN, HUXLEY and KATZ 1949, HODGKIN 1951). In frog skin such delayed rectification has not been observed at the low current densities used for the measurement of the d.c. resistance.

d.c. resistance. The 3 electrode arrangement enabled a check to be kept on this even when dilute solutions were used in 1. Even then, owing to the small distance between the E_g electrode and the skin, the high frequency impedance (20—40 Ω between the electrodes) did not much exceed the values obtained when the high frequency impedance was measured with the chamber filled with Ringer's solution in the absence of the skin (13 Ω) At least, the increase was small compared with the increase in the skin's d.c. resistance. The high frequency resistance is thus essentially a measure of the recording electrodes and the solutions on both sides of the skin, and when subtracted from the total d.c. resistance of the system the d.c. resistance of the frog skin is obtained. The high and low (approximately zero) frequency impedances can be conveniently measured by means of square current or voltage pulses (cf. HODGKIN and RUSHTON 1946, TEORELL 1946).

Square wave generator: In order to obtain square pulses of sufficiently low frequency a pulse generator was constructed which gave synchronous sine and square waves down to a frequency of 0.3 c.p.s. By the use of an output circuit with diode clippers, a very good square wave form was obtained at the lowest as well as the higher frequencies. The time constant of the whole arrangement, generator bridge and detector, was sufficiently short to give a perfect square wave form at 2000 c.p.s. when the cell, filled with Ringer's solution and without a frog skin, was tested in the bridge.

Recording method: The sine wave from the generator described was fed to the x-plates of the cathode ray tube via an amplifier and the synchronous square wave via the bridge and detector amplifier to the y-plates. In this way a standing Lissajou figure was obtained on the screen which made possible an accurate and convenient determination of d.c. resistance by compensating the maximum amplitude of the potential difference between the recording electrodes, which approached a constant value, with that across the decade resistance. The high frequency resistance was indicated by the beginning of the voltage transient and was determined most accurately by switching over to a higher frequency square pulse.

Only in exceptional cases was it necessary to use a lower frequency than 2.7 c.p.s. Polarization at the electrode E_{rp} in 2 became appreciable at a frequency of about 0.3 c.p.s. with the usual current strength of about 20 μA through the cell, but this frequency had to be used only with skins having a very high resistance when dilute solutions were used in 1. The current pulses could then be reduced to one tenth or even less of the usual strength without much decreasing the accuracy of the resistance measurements. With this current strength there was no appreciable polarization at the E_{rp} electrode in 2 even at this low pulse frequency.

The accuracy of the resistance measurements can be estimated as ± 1—2 per cent (range) of the measured resistance in the 100 Ω range, and better for skins with higher resistances, all sources of error mentioned being taken into account. The resistance bridge was frequently tested with

a precision decade resistance in place of the cell. The advantage of the bridge method described over other perhaps simpler methods was the high degree of accuracy and the fact that rapid changes in the resistance of the skin would also be followed. Further corrections for electrode and bathing fluid resistance were easily made.

f. ***Conductance measurements of the solution in 1.*** A similar bridge to that described, and the same detector, were used to measure the resistance of the solution between the two E_{rc} electrodes to a 1000 c.p.s. alternating current. A variable condenser had to be used parallel to the decade resistance to give a sharp minimum. Two sets of symmetrical resistance arms could be used alternatively in the bridge to give it the proper sensitivity for the different measuring ranges. Two 10 k Ω resistances were most frequently used. The arrangement was calibrated with NaCl solutions of known concentrations, so that when the concentration in 1 was determined by conductance measurements, it was given as the mM concentration of a NaCl solution which at the same temperature would have the same conductivity as the solution in question. The accuracy of the resistance measurements was of the order of 0.1 per cent or better in the range corresponding to concentrations of less than 20 mM of NaCl and about 1 per cent for a 120 mM NaCl solution.

The net flux of salt through the skin could be calculated from changes in the concentration of the solution in 1, if the volume in 1 was taken to be constant. Then

$$\Phi^{\mathrm{NaCl}} = 10^6 \frac{\Delta c_1 v_1}{A \cdot \Delta t} \mu\mu\mathrm{mol\ cm^{-2}\ sec^{-1}} \qquad (2.2.1)$$

where $\Delta c_1 = c_1^{t(m)} - c_1^{t(m+1)}$ is the difference between the concentrations (in mM) of the solution in 1, at the time of the m-th and $(m+1)$-th measurement and $\Delta t = t^{(m+1)} - t^{(m)}$ in seconds. (The time was always measured in minutes but in the calculations is was expressed in seconds). A is the area of the skin in cm^2 and v_1 the volume in 1 in ml.

The assumption that the solution in 1 has a constant composition requires some comment. Owing to the selective active transport of sodium through the skin, this cannot really be true. Owing to the difference in the total potential gradient through the skin for Na^+ on one side and K^+ and Ca^{++} on the other side, Ca^{++} and K^+ concentrate in 1 compared with Na^+, and the reverse is true for the solution in 2. However, the effects cannot be very pronounced for the duration of the experiments and the volumes of solutions used in 1 and 2. When due regard is given to minor changes in the composition of the electrolyte solution which will be considered below (2.3.D.f) and to temperature variations ($\pm 0.1^\circ$C), it seems safe to state that the concentration values of NaCl in 1, obtained from conductivity measurements, are correct within $\pm$ 2 per cent.

g. ***The potential-controlling circuit.*** In order to control the potential difference between the two sides of the skin when desired, a potential-controlling circuit, shown schematically in Fig. 3, was connected to the E_i electrodes. A variable electromotive force was obtained from a potentiometer coupling. There was a 200 k Ω resistance (r_3) in series to make the total resistance of the circuit large compared with the skin resistance. Thus the introduction of the potential controlling circuit did not influence the skin resistance measurements by more then about 0.1 per cent when the same concentration of Ringer's solution was used in 1 and 2 (skin resistance of the order of 200 Ω).

The E_i electrode was a platinized platinum ring with internal and external diameter of 14.5 and 15.5 mm respectively. The depth of each cell of the chamber was 8 mm. (It could be increased to 12 mm by adding a plate between the end wall and the perspex plate with in- and outflow channels but the mixing in the cell was then found from conductance measurements to be slow. In all experiments with radioactive isotopes 8 mm deep half cells were used.)

This means that the difference in the distances from the electrode to different points of the skin was 3 mm at most. The current density which had to be used to keep the potential across the skin at zero was usually of the order of 20 μA cm^{-2} of the skin, and only in exceptional cases was it necessary to use 70—80 μA cm^{-2}, when there was Ringer's solution on both sides of the skin. The specific conductance of the Ringer's solution was about 95 Ω per cm cube, and this means that a potential difference of 2 mV might be obtained between different parts of one skin surface in some cases, though it was usually less than 1 mV. The E_{rp} electrodes reduce this potential difference a little. The distance between the E_i and the E_{rp} electrodes was 1 mm. The difficulty of estimating the amout of polarization at the E_{rp} electrodes makes it impossible to say how much they reduce the potential difference. In any case, it seems certain that the potential differences due to different distances between the E_i electrodes and the various parts of the skin was not greater than about 1 mV and in most cases smaller, when zero potential was maintained across the skin. It should be mentioned in this connection that a check was made to ensure that the flow of a current in the potential-controlling circuit did not influence the resistance measurements and the polarizability of the E_{rp} electrodes towards the square pulses.

When a current was flowing trough the potential-controlling circuit, it was of importance that the potential difference measured corresponded to points in the solution near to the skin surface. To get the true potential difference across the skin a correction must then be made for the potential drop in the bathing solutions from the point where the potential is measured to the skin surface. With the arrangement described this distance was about 2 mm, and direct measurements showed that it corresponded to a potential difference of 0.2 mV per 100 μA through the cell.

A multiflex-galvanometer was used to measure the current in the circuit. It was calibrated against a precision instrument. Readings of the potential and adjustments of the current were made at least every 10th minute when a fixed voltage was used in the experiments, and during periods of rapid changes readings were made every minute. By adding the $I \times \Delta t$ values for the different periods, a good estimate of the total quantity of electricity which had flowed through the circuit was obtained.

A difficulty with the arrangement described was that current flow caused a change in the pH of the bathing solutions. This was counteracted by the use of buffered solutions and the addition of small amounts of 0.1 M HCl and NaOH solutions. In this way the pH of the solutions could usually be kept within 0.1 pH unit, and never exceeded 0.2 pH units. This was checked by frequent measurements of the pH with a microglass electrode.

Cl_2 and hypochlorite might be expected to form at the anode, though in only small quantities (cf. MACINNES 1947, p. 27, and GLASSTONE 1948, p. 1017). Repeated experiments with varying current densities of up to 500 μA through the cell during circulation of the fluids by bubbling air through the solutions as in the experiments with frog skins showed only very small amounts of Cl_2 or hypochlorite in the solutions. Titrations were made with neutral red, which is bleached by Cl_2 and hypochlorite (cf. TEORELL 1932), using a standard hypochlorite solution for comparison. The minute amounts of Cl_2 or hypochlorite does not seem to have influenced the behaviour of the skins appreciably.

h. *Radioactive tracers used in the experiments and the preparation of the solutions.*

Na^{24} was obtained from the Atomic Energy Research Establishment at Harwell, England, with an activity on arrival of 6—20 mC in 0.25 g of Na_2CO_3. It was dissolved in water and HCl, KCl, $CaCl_2$ and phosphate buffer or $NaHCO_3$ were added from stock solutions in proper quantities to give a composition corresponding to the Ringer's solution used (see below). The pH was adjusted by the addition of small amounts of 0.1 M solutions of HCl or NaOH.

Br^{82} was also obtained from Harwell as irradiated NaBr. The preparation also contained Na^{24} but owing to the short half-life (14.9 hrs) as compared with Br^{82} (34 hrs), the Na isotope decayed rapidly, and after 8 days less than 0.5 per cent of the total activity was due to Na^{24}. Solutions were prepared as in the case of Na^{24}.

The specific activity of the Br^{82} was about 2.5—0.25 mC per g NaBr during the days when the experiments were performed. The concentration of Br ions could thus be kept fairly low in the solutions and was less than 3.7 mM in all experiments but one in which it was 9 mM.

Cl^{36} was obtained from Oak Ridge National Laboratory, Tennessee. It had a specific activity of 0.2 mC per g Cl^- and was obtained as a 1.46 N HCl

solution with contaminations of less than 0.1 per cent. Its half-life is about 10^6 years.

Ringer's solutions used: Two kinds of Ringer's solutions were used in the experiments with the radioactive isotopes.

1) Ringer containing bicarbonate and with a pH of about 8.1 to 8.2. It contained 111.15 mM NaCl, 2.68 mM KCl, 3.60 mM/2 $CaCl_2$ and 3.81 mM $NaHCO_3$. Its ionic strength was 0.123 and it contained 114.9 mM Na^+ and 117.4 mM Cl^-. As a rule skins had lower resistances with this solution than with the other solution.

2) Ringer with phosphate buffer, which had a pH of 7.4 to 7.5. It was made up from 9 parts of a solution containing 111.15 mM NaCl, 3.60 mM KCl, and 3.81 mM/2 $CaCl_2$ and one part of the 0.1 M phosphate buffer made up by Na_2HPO_4 and NaH_2PO_4 in proportions 16.16/1.92. It had an ionic strength of 0.135 and contained 119.0 mM Na^+ and 105.7 mM Cl^-. It had a quite good buffering capacity. The addition of 0.1 ml 0.1 M HCl or NaOH to 10 ml of this Ringer's solution changed the pH less than 0.3 units. This solution was used in the experiments with the same solution on both sides of the skin, in which extensive use was made of the potential controlling circuit to keep the potential across the skin at zero value. With current flowing through the chamber, the buffering capacity of the solution was of importance.

Glucose was not used in these experiments, and from a large number of experiments made both with 0.1 per cent glucose in the solutions and without glucose it seems doubtful if glucose is essential for the maintainance of the isolated skin in proper condition during the experimental periods used.

The radioactive solutions for the experiments were prepared as follows. Usually the appropriate amount of the Na^{24} stock Ringer's solution was added to inactive Ringer's solution to give an activity corresponding to about $1—2 \times 10^5$ counts min^{-1} ml^{-1}. The Cl^{38} stock solution was added with an equivalent amount of 0.1 N NaOH to give a Cl^{36} activity corresponding to about 2×10^4 counts min^{-1} ml^{-1}. The pH was adjusted and checked by measurements with a microglass electrode and the solution, usually about 10 ml in volume, was ready to be added to the frog skin cell. In experiments with Br^{82} a similar procedure was adopted.

The radioactivity of the solutions did not seem to damage the skin, since the skins behaved in the same way with active or inactive solutions, to judge from the electrical measurements. Nor did a wide variation in the activity of the solutions seem to make any difference (experience from earlier experiments by the author).

i. ***Measurement of the relative activity of the radioactive isotopes.*** Samples were drawn from the radioactive solutions by means of semi-automatic Carlsberg pipettes (Levy 1936). Volumes usually of 0.1 to 0.4 ml were pipetted into small aluminium dishes with filter paper on the

bottom, and a drop of 50 per cent glucose was added. The dish was heated in an electrical oven at 105—110°C. In this way, the radioactive material was evenly distributed at the bottom of the dish (cf. USSING 1949 b).

A Geiger-Müller tube with a mica window (3—4 mg/cm^2) and a decade pulse counter (LKB scale of 100, ÅSTRÖM 1950) was used to determine the radioactivity of the preparations. As a rule, at least 2000 impulses were counted, and in many cases more than 10000 counts recorded. The counting time was always at least 2 minutes. The relative activity of the preparations varied, but as a rule less than 20000 c.p.m. were registred. Highly active preparations were allowed to decay to a suitable relative activity, and were then counted again. Most preparations had activities corresponding to a few thousand c.p.m. and less.

When measurements were performed, the starting voltage and a plateau were determined each day to control the Geiger-Müller tube. An uranium standard was frequently counted, as well as the background, which was about 18 c.p.m. The standard error of the mean background c.p.m. was 0.25—0.4 on different days. The statistical counting error of the active preparations was about 2 per cent or less (standard error).

The recorded counting rate (n) for all counting rates above 2000 c.p.m. was corrected for the resolving time of the counter to give the true counting rate (N). A calibration curve was constructed, according to the principles given by KOHMAN (1945) quoted from SIRI (1949), of the form

$$N = n\,k_1 + n^2\,k_2 + n^3\,k_3 .$$

It was checked by measurements on samples of known relative activity and also by following the decay of Na^{24} and Br^{82}. Applying this correction, the counter was found to give correct values at counting rates of up to 50000—70000 c.p.m.

In this connection it should be mentioned that the half life of Na^{24} seemed to be a little longer than the 14.9 hrs given in the Harwell Catalogue. 15.0 hrs seemed to fit the experimental results better and was the figure used when corrections for decay were made. This may be due to some small amount of impurity, and a small »rest» activity was usually obtained from strong preparations. When both Na^{24} or Br^{82} had decayed to less than 1 per cent of the remaining Cl^{36} activity, the preparation was recounted. The counting rate of the remaining Cl^{35} (N_t^{Cl36}) was subtracted from the first counting rate ($N_t^{Na24+Cl39}$) to give the N_t^{Na24}. After correction for decay the counting rate of the Na^{24} activity present at a chosen zero time, usually corresponding to the beginning of the experiment, N_0^{Na24}, was obtained.

Self-absorption in the preparations was neglected. The amount of substance (NaCl and glucose) was small and usually of the same order of magnitude. In the case of Cl^{36}, which had the lowest energy radiation, preparations were made by diluting the strong stock solution in distilled water

and in Ringer's solution. No certain difference was found, and the error due to self-absorption therefore seems to have been negligible.

The coefficient of variation of a single determination of relative radioactivity was 4 per cent when different pipettes were used and relative activities in a wide range were determined. This error includes possible errors introduced in connection with the correction for the resolving time of the counter and for decay. When the same pipette was used for determinations on the same radioactive solution, the coefficient of variation of a single determination did not differ appreciably from the statistical counting error.

j. ***Calculation of the flux of ions from tracer experiments.*** The experiments were generally performed so that the radioactive solution was added to one side of the skin and samples of 0.4 ml were drawn from the other side at intervals of 15 to 30 minutes. Immediately after a sample was taken, the same volume of inactive solution was returned. From the side of the radioactive solution, samples usually of 0.1 ml were drawn less frequently, at about 2 hours intervals. This solution was not renewed, neither with inactive nor with radioactive solution.

In this way, the activity of the solution into which the radioactive isotopes diffused was kept low, and its maximum activity was usually less than 1—2 per cent of the specific activity of the highly radioactive solution, even in the experiments with diuretics, where the permeability of the skin sometimes increased a good deal. In some experiments it was far lower. Thus back-diffusion could usually be neglected, especially in the cases when the flux of the ion in question was considerably greater from the radioactive solution than in the reverse direction, as estimated from the total potential difference of the ion across the membrane. The maximum back-diffusion of radioactive ions in all the experiments was calculated from the total activites of the ions in the two solutions on either side of the skin. It was usually less then 2 per cent of the diffusion in the direction of measurement and only in some experiments with diuretics did it amount to a little more then 3 per cent when the flux was measured from 2 to 1.

Assuming the radioactive ions to behave in the same way as inactive ones, the total flux of an ion species B^i in one direction was usually calculated according to the following formula.

$$\Phi_1^i = 10^6 \cdot \frac{\{v_2^{t(n+1)} \cdot N_2^{t(n+1)} - (v_2^{t(n)} - q_2) \cdot N_2^{t(n)}\}}{q_2 \cdot \bar{N}_1 \cdot \Delta t \cdot A} \frac{c_1^i q_1}{} \mu\mu\text{mol cm}^{-2}\,\text{sec}^{-1}. \tag{2.2.2}$$

Here $v_2^{t(n)}$ and $v_2^{t(n+1)}$ are the volumes of solution 2 in ml at the time when the n-th and $(n+1)$-th sample were drawn and $N_2^{t(n)}$ and $N_2^{t(n+1)}$ are the relative activities in c.p.m. of the isotope of the ion B^i in the same samples. $\bar{N}_1$ is the relative activity of the isotope of the ion B^i

in the samples drawn from 1, interpolated to correspond to the mean activity during the period $\Delta t = t^{(n+1)} - t^{(n)}$ in seconds. All activities are corrected for decay. q_1 is the volume in ml of the pipette used to measure the samples in 1. q_2 is the volume in ml of the pipette used in 2. c_1^i is the concentration of the ion in question in 1 in mM. A is the area of the skin in cm^2. If the flux from 2 to 1, $-\Phi_2^i$, is to be determined, the indices 1 and 2 change places in the formula.

In some exceptional cases when a sample was drawn from the solution of low activity some time between $t^{(n)}$ and $t^{(n+1)}$ for other purposes than activity measurements, corrections were made for this, assuming linear increase in the concentration of radioactive ions during the period Δt.

In a few cases when the flux values of adjacent, usually short, periods differed markedly and gave improbable values for G^{Cl}, G^{Na} and φ_a^{Na} the mean flux values of the two periods were used. In some exceptional cases the activity value of a sample was corrected by interpolation according to the following principle: If a curve was drawn with the activity of the different samples plotted against time, usually a smooth curve was obtained. In some experiments one point deviated more or less markedly from the curve, probably owing to some experimental error in the sampling or handling of the preparation, since the activities of both the isotopes, e.g. Na^{24} and Cl^{36}, usually deviated in the same way. The curve was used for graphical interpolation.

Chloride titrations to check the concentrations of the solutions were performed electrometrically.

pH measurements were made by means of a microglass electrode requiring only 0.1 ml of solution for a determination.

2.3. Experiments made to study the active transport potential of sodium ions and the transfer of sodium chloride through isolated frog skin.

A. Some general assumptions in the treatment of the experimental data.

The following is an account of a series of experiments in which »normal» isolated frog skins, i.e. skins not exposed to the action of drugs, were studied with the methods outlined in the previous sections. Some simplifying assumptions have been made to make it possible to apply the equations derived in the theoretical part directly to the experimental system.

The assumptions are:

1) The skin is, as a first approximation, supposed to behave as a homogeneous uncharged membrane (see 1.3.b. and 1.4).
2) Only Na ions are transported actively in the skin, and Cl ions are supposed to be passive.
3) The relatively small amounts of other ions than Na^+ and Cl^- in the Ringer's solution used may be neglected. It is also assumed that no ions diffuse out from the membrane itself.
4) The influence of the flow of water through the skin on the transfer of ions may be neglected.

The first three assumptions will be discussed later in connection with the experimental results. The fourth assumption requires some comment, which will be made here, as it is essentially based on data assembled from literature and on theoretical considerations.

In intact frogs, v. Hevesy, Hofer and Krogh (1935) found a net uptake of water, Φ^w, of 5 mg cm^{-2} hr^{-1} when the skin was bathed with distilled water. Sawyer (1951), working with isolated frog skin, found $\Phi^w = 4.73$ μl cm^{-2} hr^{-1} with distilled water on the outside. Under conditions similar to those of some of the present experiments with a dilute solution in 1, Fuhrman and Ussing (1951) found $\Phi^w = 7$ μl cm^{-2} hr^{-1} with Ringer's diluted tenfold on the outside of the skin. Owing to their technique, their values cannot be very exact, but they are of the same magnitude as those reported by others.

In a few preliminary experiments by the author, Φ^w was studied in skins with a dilute solution on the outside. The frog skin chamber described in 2.2.b was used. The outflow tube of the half cell 1 was connected with a horizontal capillary tube, which made it possible to read volumes to the nearest μl. The inflow tube was closed and the changes in volume of the fluid in the half cell 1 could be determined by pressing the skin with constant pressure against the sieve at each reading.

For three skins with a NaCl solution of about 5 mM in 1, Φ^w was found to vary between 3.1 and 4.7 μl cm^{-2} hr^{-1}.

It is important in this connection, however, that even with solutions of the same concentration on either side of the skin, the net flux of water from the epithelial side to the chorium side is of about the same magnitude. As mentioned earlier,

RIED (1892) observed this transport, and an analysis of his experimental data reveals that in the earlier part of his experiment about 3—4 μl cm^{-2} hr^{-1} passed through the skin.

Similar experiments were carried out by HUF (1936 b) and, in spite of the fact that the skin in some of these experiments had to work against a hydrostatic pressure of 2 cm of water, similar figures were reported. Later, HUF and collaborators have used the skin bag method to determine the active salt and water transport with Ringer's on both sides.

From three experiments in which the skin bags were bathed with Ringer's solution of pH 7.3 on each side HUF, PARRISH and WEATHERFORD (1951) give some data which make it possible to calculate a mean $\Phi^w = 3.1 - 3.8$ μl cm^{-2} hr^{-1} during a 12 hour period.

In order to estimate the effect of the flow of water on the diffusion of ions through the frog skin, both with Ringer's solution on either side and with a dilute solution on the outside, it was assumed that:

a) The net flux of water, Φ^w, is 5 μl cm^{-2} hr^{-1}, which is probably a rather high figure in the case of a solution of the same concentration on either side of the skin.

b) The skin permeability of H_2O is the same as that of D_2O, which was determined by v. HEVESY, HOFER, and KROGH (1935). They found that it will take 100 170 days for 1 mol of D_2O to pass through 1 cm^2 of skin when the concentration difference is 1 M.

c) The concentration of water in Ringer's solution is 55.3 M and 55.5 M in pure water (cf. USSING 1950). It may then be calculated by using eqn. (1.8.3) that the water flux ratio $-\Phi_2^w / \Phi_1^w$ is about 0.98.

If the diffusion coefficients of Na and Cl ions are calculated from their mobilities and the diffusion coefficient of NaCl (1.39×10^{-5} cm^2 sec^{-1} at 20°C from International Critical Tables), and the diffusion coefficient of D_2O is taken as 2.2×10^{-5} cm^2 sec^{-1} — interpolated from the values given by ORR and BUTLER (1935) and TEMKIN (1935) — the value of ξ_{iw}^i can be obtained from eqn. (1.9.2), or (1.9.3). For both Na and Cl ions it is about 1.01 or less. If the same calculation is made

without the Ussing correction, i.e. the square of the activity ratio in eqn. (1.8.2), ξ^i_{ac} is about 1.02. It thus seems improbable that the flow of water will change the flux ratio $-\Phi^i_2/\Phi^i_1$ by more than about 1 per cent, and this factor may therefore be neglected.

B. Experiments with solutions of the same composition on both sides of the skin.

The methods used in this series of experiments were as outlined in the previous sections. Ringer's solution with a phosphate buffer of pH = 7.5 was used on both sides of the skin. The total fluxes of Na and Cl ions were determined simultaneously, either from 1 to 2 or in the reverse direction by means of their isotopes (Na^{24} and Cl^{36}). In some experiments only the Cl^{36} flux was measured. The electrical potential across the skin, φ, and its total conductance, G, were also recorded. During a later period of the experiment the potential-controlling circuit was often used to keep the potential across the skin at zero value, and during this time the current through this circuit was also recorded.

By the methods described in Chap. 1, G^{Cl} was calculated from eqn. (1.3.14) or (1.3.15), G^{Na}_{aI} from (1.3.4), φ^{Na}_{aI} from (1.5.4) or (1.7.1), φ^{Na}_{aII} from (1.7.2) or (1.7.3), G^{Na}_{aII} from (1.4.3) after evaluating the active transport term ξ^{Na}_a from the φ^{Na}_{aII} value by means of eqn. (1.2.8). The activities of the Na and Cl ions used in the equations were calculated from the activity coefficients obtained from the tables given by HARNED and OWEN (1950), with due regard to the ionic strength of the Ringer's solutions. In order to make possible a comparison between the G values and the partial conductances, the resistance of the skin was measured frequently, especially during periods when it changed. The mean G value for each 15—30 minute period corresponding to the interval $\Delta t = t\,(n+1) - t\,(n)$, see 2.2.j, was obtained by the graphical integration of the G curve with respect to time. The results of some typical experiments are presented in Figs. 4 and 5.

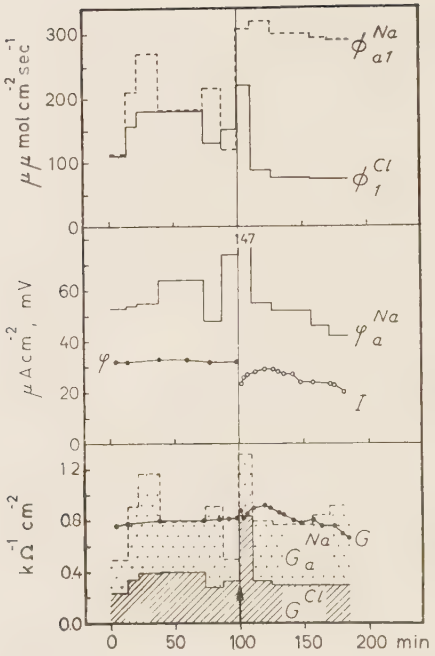

Fig. 4. Typical experiment, 7.1, in which the inward fluxes of Na and Cl ions through a frog skin were measured, first during a period when the skin was open-circuited, and later when it was short-circuited and φ maintained at zero by means of the potential-controlling circuit. In the upper part of the figure are the flux values Φ^{Na}_{a1} and Φ^{Cl}_{1}. Below are given the active transport potential, φ^{Na}_{a}, calculated as the mean of φ^{Na}_{aI} and φ^{Na}_{aII}, the potential across the open-circuited skin, φ, and I, the current forced through the skin by means of the potential-controlling circuit in the short-circuited skin ($\varphi = 0$). In the lowest part of the figure the electrically measured conductance G and the partial conductances, G^{Cl} (shaded area) and G^{Na}_{aII} (dotted area) are given. The dotted line which represents the sum of G^{Na}_{aII} and G^{Cl} appears to correspond fairly well with the electrically measured G. The arrow indicates where the potential-controlling circuit was switched in. Ringer's solution on both sides.

a. ***Experiments made to test the assumption that Cl ions are passive.*** The assumption that Cl^- behaves like a passive ion and that only Na ions are actively transported is of great importance in the calculations. The experimental results will

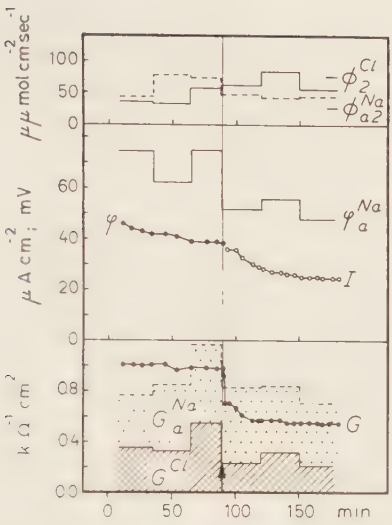

Fig. 5. Experiment 9.3. A similar experiment to that described by Fig. 4. The fluxes were measured outwards through the skin.

therefore be analyzed in order to see how they sustain the assumption.

Open-circuited skins. The flux of Cl ions was measured in some skins from 1 to 2 and in others from 2 to 1. If a sufficient number of skins were investigated and the mean flux in either direction compared in skins having a similar φ, the mean flux values would be expected to satisfy eqn. (1.2.1.) fairly well if Cl^- were a passive ion. The number of experiments performed was not sufficient to justify the use of this method. However, G for the skin was measured at the same time as the flux. Now, if Φ_1^{Cl} of one skin is compared with Φ_2^{Cl} of another skin having nearly the same G and the same φ, fairly close agreement might be expected even with a smaller number of skins. Deviations from eqn. (1.2.1) may be explained by the fact that G^{Cl} is not quite a constant fraction of G.

From 13 experiments four pairs of skin were selected which fulfilled the conditions mentioned fairly well, and the results for these skins are given in Table 1.

Table 1.

Flux of Cl ions inward through frog skin Φ_1^{Cl} compared with the outward Cl flux Φ_2^{Cl} in skins with similar total conductance G and potential difference φ.. Ringer's solution on either side. Mean values from each experiment. For further explanation see text.

Exper. no.	n	φ mV	G $k\Omega^{-1}\,cm^{-2}$	Φ_1^{Cl}	$-\Phi_2^{Cl}$	$\frac{-\Phi_2^{Cl}}{\Phi_1^{Cl}}$	ξz^i	$\frac{-\Phi_2^{Cl}/\Phi_1^{Cl}}{\xi z^i}$
10.3	5	34	1.44	327		0.30	0.28	1.1
12.1	3	31	1.24		99			
8.1	4	39	1.21	347		0.13	0.21	0.6
9.3	3	40	1.19		44			
13.1	7	48	0.96	236		0.22	0.15	1.4
8.2	2	44	0.93		51			
14.1	4	50	0.73	137		0.23	0.17	1.4
15.1	3	42	0.74		32			
Mean		41	1.06	262	57	0.22	0.20	1.1

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}.

n is the number of 15—30 minute periods included in each experiment

As seen from Table 1, the flux ratio found deviates rather much from the value expected from the potential difference in some cases, but the mean quotient $(-\Phi_2^{Cl}/\Phi_1^{Cl}) : \xi z^i$ is 1.1 and, taken together, the experiments seem to corroborate the assumption that Cl^- behaves like a passive ion, i.e. follows the condition imposed by eqn. (1.2.1). More experiments of this kind are required to give the assumption a high degree of probability.

Short-circuited skins with potential kept at zero. During periods when the skin potential is kept at zero, eqn. (1.2.1) requires no net flux of a passive ion, i.e. $-\Phi_2^{Cl}/\Phi_1^{Cl} = 1$. The mean Φ_1^{Cl} from 5 experiments was compared with the mean Φ_2^{Cl} from 5 other experiments. The mean flux values found were: Φ_1^{Cl} 67.6 and $-\Phi_2^{Cl}$ 71.2 $\mu\mu$mol cm^{-2} sec^{-1} and the $-\Phi_2^{Cl}/\Phi_1^{Cl}$ quotient was 1.05.

The method in the preceding section was also used. Selected pairs of skins with a similar G were taken from the two groups, and the skins of each pair were compared. Four such pairs were obtained, the results being summarized in Table 2.

Table 2.

Flux of Cl ions inward through frog skin compared with the outward flux in a skin with similar total conductance G during periods when the skin potential was kept at zero by means of the potential-controlling circuit. Ringer s solution on either side. Mean values from each experiment.

Exper. no.	n	Φ_1^{Cl}	G	Exper. no.	n	$-\Phi_2^{Cl}$	G	$\frac{-\Phi_2^{Cl}}{\Phi_1^{Cl}}$
7.1	4	80	0.81	9.3	3	67	0.79	1.1
7.3	4	71	0.53	8 2	3	61	0 51	0.9
11.1	4	55	0.70	15.2	4	55	0.69	1.0
14.2	4	60	0.65	12.2	4	69	0.66	1.2
Mean		67	0.67	Mean		63	0.66	1.0

Flux values in $\mu\mu$mol cm^{-2}, see.$^{-1}$, G *in* kΩ^{-1}, cm^{-2}.
n is the number of 15—30 minute periods included in each experiment.

Combined with the results in the case where there was a potential difference across the skin, *the results sustain the assumption that* Cl^- *behaves like a passive ion in frog skin* under the conditions of the present experiments.

b. ***Experiments with*** Cl^{36} ***and*** Br^{82} ***in combination to test whether these ions behave as passive ions.***

Experiments in which the fluxes of Cl^{36} *and* Br^{82} *were measured in the same direction.* These experiments were made in order to compare the two ions. The Br flux values were calculated as if Br ions occurred in the same concentration and had the same activity as the Cl ions. As they generally behave rather similarly in living cells, it might be expected that their

flux values would be rather similar. The experiments verified this assumption in most cases. Table 3 gives the ratio Φ^{Cl}/Φ^{Br}, which appears to be generally a little less than 1. If the ions diffused in free solution, it might be expected that the flux ratio would equal the ratio between their mobilities u^{Cl}/u^{Br}, which is 0.97. This happens to agree quite well with the mean ratio in Table 3. Slightly lower ratios were obtained in most of the

Table 3.

Quotient of Cl^- and Br^- fluxes measured in the same direction through frog skin during periods with no current in an outer circuit, i.e. with the spontaneous resting potential difference across the skin. Only in one experiment (15.1) were the fluxes measured outward through the skin. Mean values from each experiment.

Exper. no.	n	$\frac{\Phi^{Cl}}{\Phi^{Br}}$
12.3	7	1.26
13.1	9	0.89
14.1	5	0.87
15.1	3	0.86
Mean		0.97

n is the number of 15—30 minute periods included in each experiment.

experiments. The results seem to indicate that Br^{82} can in many cases be used as a good substitute for Cl^{36} in experiments on frog skin.

In two experiments in which the fluxes of Cl and Br ions were measured outward through the skin, there was a marked difference between the two fluxes when the potential-controlling circuit was used to keep φ at zero. In both these experiments the $-\Phi_2^{Br}$ was far lower than the $-\Phi_2^{Cl}$ for a considerable time, about 1 hour. Then they seemed to approach each other. It has not been possible to explain this fact, but it may be due to a non-steady state of Br ions in the skin.

Experiments in which the fluxes of Cl^{36} and Br^{82} were measured in opposite directions. Two such experiments were performed. In one the active Br^{82} solution was on the outside, and in the other on the inside of the skin. The active Cl^{36} solution was on the opposite side of the skin. Samples of 0.4 ml were drawn from each side at intervals of about 30 minutes. In these experiments there is a relatively large uncertainty in the determination of the Br flux. This involved the subtraction of two large numbers, $N_t^{Br+Cl} - N_t^{Cl}$, to obtain the relatively small number N_t^{Br} which was due to the activity of Br^{82} in the solution into which Br^{82} diffused. The Br flux values also varied rather much between the different 30 minute periods, but the mean of some periods seems to give fairly correct values. The results are summarized in Table 4.

Table 4.

Fluxes of Cl^- and Br^- measured in opposite directions in the same open-circuited skin. Mean values from each experiment. For further explanation see text.

Exper. no.	n	φ mV	Φ_1^{Cl}	$-0.97\,\Phi_2^{Br}$	$\frac{-0.97\,\Phi_2^{Br}}{\Phi_1^{Cl}}$	ξz^i	$\frac{-0.97\,\Phi_2^{Br}/\Phi_1^{Cl}}{\xi z^i}$
11.1	4	55	159	25	0.16	0.11	1.4

Exper. no.	n	φ mV	$0.97\,\Phi_1^{Br}$	$-\Phi_2^{Cl}$	$\frac{-\Phi_2^{Cl}}{-0.97\,\Phi_1^{Br}}$	ξz^i	$\frac{-\Phi_2^{Cl}/0.97\,\Phi_1^{Br}}{\xi z^i}$
12.1	3	31	317	99	0.31	0.29	1.1

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}.
n is the number of 15—30 minute periods included in each experiment.

As the bromide ions may be expected to have a little higher mobility in the skin than the chloride ions (cf. the preceding section), all the Φ^{Br} values have been multiplied by 0.97, i.e. the ratio between the mobilities in water of chloride and bromide. The Br values corrected in this way are supposed to

be a measure of the chloride flux in the same direction. As seen from Table 4, the values agree satisfactorily with the assumption that Cl and Br ions are passive.

c. ***The Na ion as carrier of the current produced by the skin.*** It has been shown by USSING and ZERAHN (1951) that the

Table 5.

Fluxes of Na and Cl ions inward and outward through frog skins during periods when the potential was kept at zero by means of the potential-controlling circuit. The current flowing through the potential-controlling circuit I, the total conductance G and the calculated active sodium transport potential φ_a^{Na} are also given. Ringer's solution on either side. Mean values from each experiment.

Inward fluxes						
Exper. no.	n	Φ_{a1}^{Na}	Φ_2^{Cl}	I/F	φ_a^{Na} mV	G $k\Omega^{-1}\,cm^{-2}$
7.1	4	303	80	254	49	0.81
7.3	4	137	71	88	35	0.53
10.1	7	173	92	113	53	0.58
Mean		204	81	152	46	0.64

Outward fluxes						
Exper. no.	n	$-\Phi_{a2}^{Na}$	$-\Phi_2^{Cl}$	I/F	φ_a^{Na} mV	G $k\Omega^{-1}\,cm^{-2}$
9.3	3	43	67	287	52	0.79
8.2	3	41	61	170	52	0.51
9.1	3	108	105	276	34	1.20
Mean		64	78	244	46	0.83

Flux values in $\mu\mu$mol $cm^{-2}\,sec^{-1}$, I/F in $\mu\mu$ $F\,cm^{-2}\,sec^{-1}$.
n is the number of 15—30 minute periods included in each experiment.

current produced by short-circuited »normal» isolated frog skin, with Ringer's solution of the same concentration on both sides, is carried entirely by the Na ions, and that current expressed in equivalents of gram ions (F) per unit time is the same as the net flux of Na ions. In order to test whether the results of this investigation agree with the experimental results reported by USSING *et al.*, the experiments in which Na and Cl fluxes were measured from 1 to 2 and from 2 to 1 in the short-circuited skin were compared. The results from these experiments are given in Table 5.

In three skins the flux was measured from 1 to 2, and in three other skins in the opposite direction. The mean Φ_1^{Cl} and $-\Phi_2^{Cl}$ from the two groups was found to agree rather well, better than might be expected in view of the small number of skins in this group and the relatively large variation within the groups as estimated from the total conductance values. The difference between the Na fluxes in opposite directions, $\Phi_1^{Na} + \Phi_2^{Na} = 140$ $\mu\mu$mol cm^{-2} sec^{-1} was found to be of the same order of magnitude as the current flowing through the skin, $I/F = 198$ $\mu\mu F$ cm^{-2} sec^{-1}. If two skins were compared (exper. 7.1 and 9.3) which had about the same G and produced about the same current, a still better agreement was obtained: $\Phi_1^{Na} + \Phi_2^{Na} = 260$ $\mu\mu$mol cm^{-2} sec^{-1} and $I/F = 271$ $\mu\mu F$ cm^{-2} sec^{-1}.

d. ***Total and partial conductances.*** The results of the electrical conductance measurements and the calculations of the partial conductances for different experimental periods in »normal» isolated frog skin are summarized in Table 6.

It is evident from the quotient $G/(G_{all}^{Na} + G^{Cl})$ that the sum of the partial conductances of Na and Cl ions agrees quite well with the electrically measured conductance.

Table 6.

Mean electrically measured total conductance G, partial conductances and ratios between them for different groups of skins with Ringer's solution on either side.

Experimental arrangement The values from each skin are the mean of 3—7 periods, each of 15—30 minutes	G	G^{Cl}	$\frac{G_{aI}^{Na}}{G^{Cl}}$	$\frac{G_{aII}^{Na}}{G^{Cl}}$	$\frac{G}{G_{aII}^{Na}+G^{Cl}}$
1) *Open-circuited skins* ($\varphi \neq 0$).					
a) Mean values from 4 skins (7.1, 8.1, 10.1, 10.3). Partial conductances calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	1.13	0.58	1.0	1.0	1.00
b) Mean values from 3 skins (8.2, 9.1, 9.3). Partial conductances calculated from Φ_{a2}^{Na} and Φ_2^{Cl}	1.07	0.45	1.6	1.5	1.04
c) Mean of the values in a) and b) (7 skins)	1.10	0.51	1.3	1.3	1.02
d) Mean values from 8 skins, including those in a). Partial conductance from Φ_1^{Cl}	0.97	0.43	1.6	—	
e) Mean values from 5 skins, including those in b). Partial conductance from Φ_2^{Cl}	1.04	0.48	1.4	—	
f) Mean of the values of d) and e) (13 skins)	1.00	0.45	1.5	—	–
2) *Short-circuited skins* ($\varphi = 0$).					
a) Mean values from 3 exper. (7.1, 7.2, 10.1). Partial conductances calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	0.64	0.31	1.1	1.4	0.92
b) Mean values from 3 skins (8.2, 9.1, 9.3). Partial conductances calculated from Φ_{a2}^{Na} and Φ_2^{Cl}	0.83	0.30	1.8	2.0	0.92
c) Mean of the values of a) and b) (6 exper.)	0.74	0.31	1.5	1.7	0.92
d) Mean values from 5 skins, including those in a). Partial conductance from Φ_1^{Cl}	0.65	0 26	2.0	—	—
e) Mean values from 5 skins, including those in b). Partial conductance from Φ_2^{Cl}	0.77	0.30	1.7		–
f) Mean of the values of d) and e) (10 skins)	0.71	0.28	1.9		
Mean of all experiments in open- and short-circuited skins	0.90	0 39	1.5	1.4	0.97

For the meaning of G_{aI}^{Na} and G_{aII}^{Na}, cf. 2.3.B, p 57.

G values in $k\Omega^{-1}\,cm^{-2}$.

e. ***The active transport potential.*** The values of the active transport potential q_a^{Na} are given in Tables 5 and 7 as the mean of q_{aI}^{Na} and q_{aII}^{Na}. The two different ways of calculating the q_a^{Na} values give similar results. The mean q_{aI}^{Na}/q_{aII}^{Na} quotient from 7 experiments with open-circuited skins is 1.1 and for 6 experiments with short-circuited skins it is 1.3. The quotient is unaffected by the direction in which the fluxes were measured. The first 15—30 minute period after the potential-controlling circuit was switched in was usually omitted from the calculation of the mean values for each experiment, as in many cases improbable values were obtained from this period (cf. Fig. 4), probably due to a non-steady state of the skin. With Ringer's solution on both sides of the skin, the active transport potential is about 75 mV in the open-circuited skin (Table 7.). It seems generally to be lower, about 50 mV, in the short-circuited skin (cf. Table 5). In many cases the q_a^{Na} seems more or less independent of q, being about the same for open-circuited and short-circuited skins (Fig. 4).

f. ***The net transport of salt through the skin.*** The net transport of salt, Φ^{NaCl}, was calculated from eqn. (1.5.5) for the periods when the skin was open-circuited, i.e. when no current was flowing in the potential-controlling circuit. For these calculations, it is only necessary to determine G^{Cl} and G. This was done in experiments on 13 frog skins corresponding to measurements during 3—7 periods of 15—30 minutes for each skin. The mean Φ^{NaCl} of the 13 skins was found to be 178 ± 14.5 $\mu\mu$mol cm^{-2} sec^{-1} (mean $\pm$ standard error of the mean).

Another way of estimating the net transport of salt was also used. The Φ_{a1}^{Na} and Φ_1^{Cl} values from experiments in some skins were compared with the Φ_{a2}^{Na} and Φ_2^{Cl} values obtained from experiments with other frog skins during periods when the skins were open-circuited, i.e. when they had their spontaneous potential. Skins with as far as possible similar G and q were chosen. Unfortunately, there were few pairs of skins closely comparable in this respect. The results are summarized in Table 7.

Table 7.

Fluxes of Na and Cl ions inward and outward through frog skins during periods when no current flowed in an outer circuit. The potential difference φ, the calculated active sodium transport potential φ_a^{Na}, and the electrically measured conductance G are also given. Ringer's solution on either side. Mean values from each experiment.

Inward fluxes						
Exper. no.	n	Φ^{Na}_{a1}	Φ^{Cl}_{1}	φ mV	φ_a^{Na} mV	G kΩ^{-1} cm^{-2}
7.1	4	197	160	32	60	0.81
8.1	4	318	347	39	92	1.21
10.1	4	240	258	30	65	1.07
Mean		252	255	34	73	1.03

Outward fluxes						
Exper. no.	n	$-\Phi^{Na}_{a2}$	$-\Phi^{Cl}_{2}$	φ mV	φ_a^{Na} mV	G kΩ^{-1} cm^{-2}
8.2	2	73	51	44	86	0.93
9.1	3	113	38	45	73	1.09
9.3	3	66	44	40	71	1.19
Mean		84	44	43	77	1.07

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}.

n is the number of 15—30 minute periods included in each experiment.

When the net flux of Na and Cl ions respectively are calculated from the mean total flux values of this group it is found that Φ^{Cl} (211 $\mu\mu$mol cm^{-2} sec^{-1}) is a little greater than Φ_a^{Na} (168 $\mu\mu$mol cm^{-2} sec^{-1}). One would rather expect the contrary (cf. HUF *et al.* 1951). (According to the simplifying assumptions they should be equal). The divergence is probably due to the fact that the skins were not closely similar (φ, for instance is higher in the "Φ_2 skins"). The mean of Φ_a^{Na} and Φ^{Cl} is 189 $\mu\mu$mol cm^{-2} sec^{-1}, and if the Φ^{NaCl} is calculated from eqn.

(1.5.5) the mean value from the same experiments is 194 $\mu\mu$mol cm^{-2} sec^{-1}. This figure agrees satisfactorily with the above mentioned Φ^{NaCl} value obtained from a larger number of skins by eqn. (1.5.5).

C. Experiments with solutions of different concentration on the two sides of the skin.

In these experiments the solution in 1 was a diluted Ringer's solution containing about 7—9 mM NaCl (and the isotopes Na^{24} and Cl^{36}). The solution in 2 was a Ringer's solution with bicarbonate of pH 8.1—8.2. Φ_{a1}^{Na} and Φ_1^{Cl}, q and G were recorded as described. From these data the values of the partial conductances and the active transport potential were calculated as described in 2.3.B. The conductance of the solution in 1 was measured frequently, and changes in its concentration could be followed. These experiments thus provided an opportunity to study the active transport potential working against a chemical and an electrical potential. In the experiments recorded here the active transport potential balanced the other two potentials fairly well. By means of the method already described for measuring Φ^{NaCl} from changes in the concentration in the solution in 1, and the method for estimating the same flux from radioactive measurements and eqn. (1.5.5), the results of two independent methods for determining Φ^{NaCl} could be compared.

A difficulty is encountered when Φ^{NaCl} through the skin is to be estimated from the changes in the concentration in 1 by means of (2.2.1). This equation is only valid when there is no net flux of solvent through the skin ($\Phi^w = 0$). This is not so in this case, as it has been shown by many investigators that there is a net transfer of fluid from 1 to 2 (cf. 2.3.A).

Although it was not measured in the experiments reported here, it seems fair to assume a net flux from 1 to 2, Φ^w, of 5 μl cm^{-2} hr^{-1} = 1.39 $\mu\mu$l cm^{-2} sec^{-1}. Using this figure to correct the Φ^{NaCl} from eqn. (2.2.1), it is found that the Φ^{NaCl} values should be increased by $1.39 \times c_1$ $\mu\mu$mol cm^{-2} sec^{-1} to

get the true net flux of NaCl. It does not change the absolute value very much, the increase being about 10 $\mu\mu$mol cm^{-2} sec^{-1} for the present experiments.

The results of a typical experiment of this kind are given in Fig. 6. In this experiment the potential was »clamped during a short period by the use of the potential-controlling circuit. Other experiments which illustrate the behaviour of

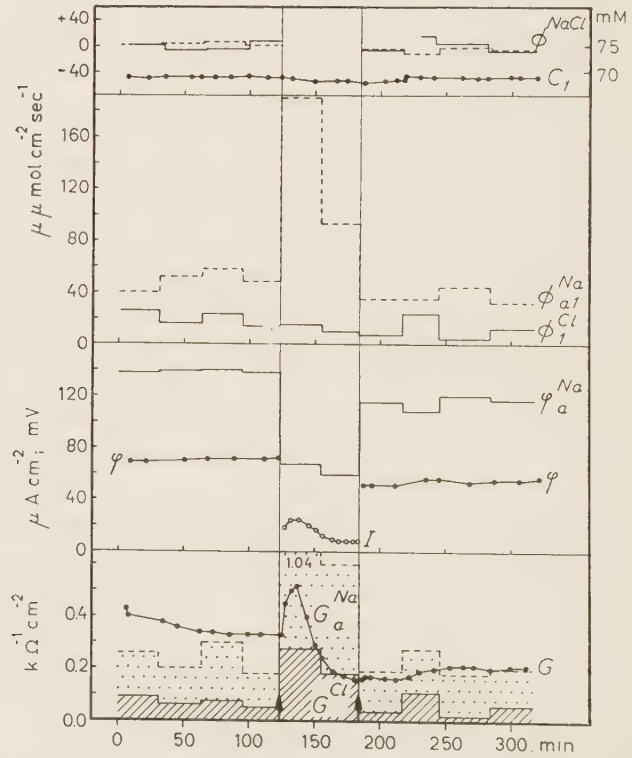

Fig. 6. Experiment 5.1. The fluxes of Na and Cl ions were measured inward through the frog skin with dilute Ringer's solution of concentration c_1 on the outside. Ringer's solution on the inside. In the highest part of the figure are given the net flux of NaCl, Φ^{NaCl}, calculated according to eqn. (1.5.5), dotted line, and from eqn. (2.2.1), full line. The small jump in the c_1 curve at about 220 minutes is due to the addition of a small amount of NaOH to adjust the pH. The rest of the figure is in accordance with Fig. 4 . At the first arrow the potential controlling circuit was switched in to keep φ at zero, at the second arrow the skin was again open-circuited.

»normal» isolated frog skin under similar conditions are presented in Figs. 12, 16 and 18, in connection with experiments with mersalyl, aminophylline and CO_2.

a. ***Conductance values.*** Values from »normal» skins are summarized in Table 8. It is evident from the table that there is

Table 8.

Total and partial conductances from the experiments with a dilute solution (about 7 mM) on the outside (1) and Ringer's solution on the inside (2) of the skin. Further data from these experiments are given in Table 9. Mean values from each experiment. Open-circuited skins.

Exper. no.	n	G $k\Omega^{-1}\ cm^{-2}$	G_a^{Na} $k\Omega^{-1}\ cm^{-2}$	G^{Cl} $k\Omega^{-1}\ cm^{-2}$	$\frac{G_{aI}^{Na}}{G^{Cl}}$	$\frac{G_{aII}^{Na}}{G^{Cl}}$	$\frac{G}{G_{aII}^{Na}+G^{Cl}}$
5.1	4	0.353	0.166	0.069	4.3	2.5	1.6
5.2	3	0.200	0.157	0.065	4.0	3.8	0.9
6.1	3	0.224	0.178	0.093	1.5	1.9	0.5
6.2	3	0.214	0.127	0.066	2.3	2.0	1.1
Mean		0.248	0.157	0.073	3.0	2.5	1.0

n is the number of 15—30 minute periods included in each experiment.

For the meaning of G_{aI}^{Na} and G_{aII}^{Na}, cf 2.3.B, p 57.

a good agreement between the conductance measured electrically, G, and the sum of the partial conductances G_a^{Na} and G^{Cl}. It is noteworthy that in these experiments the G_a^{Na} is considerably higher than the G^{Cl}, the mean ratio between them being as high as 2.5—3. It is higher than the corresponding ratio for the skins investigated with Ringer's solution on the two sides of the skin, where the mean ratio was 1.4—1.5, and no value exceeded 2.0.

b. ***The active transport potential and some flux values.*** Some other data from the same experimental periods as in the preceeding section are summarized in Table 9. η_a^{Na} is seen to

Table 9.

Mean flux values inward through the skin in the same experiments as in Table 8. The concentration range in mM NaCl of the solution on the outside c_1, the calculated active sodium transport potential φ_a^{Na} and the net transport of salt, Φ^{NaCl}, are also given. The Φ^{NaCl} calculated from eqn. (2.2.1) is not corrected for the net flux of water through the skin and should be about 10 $\mu\mu$mol cm^{-2} sec^{-1} greater (cf. text). No current in an outer circuited.

Exper. no.	n	φ mV	c_1 mM	Φ_{a1}^{Na}	Φ_1^{Cl}	φ_a^{Na}	Φ^{NaCl} from eqn. (2.2.1)	Φ^{NaCl} from eqn. (1.5.5)
5.1	4	70	(6.90 – 6.91)	50	20	138	± 0	+ 2
5.2	3	55	(6.83 – 6.90)	37	13	115	+ 1	— 8
6.1	3	47	(7.00 – 7.16)	38	16	101	— 18	— 19
6.2	3	44	(7.54 – 7.55)	38	11	101	— 9	— 14
Mean		54		41	15	114	— 6	— 10

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}.

n is the number of 15—30 minutes periods included in each experiment.

be generally higher than for the skins with Ringer's solution on each side. Φ_{a1}^{Na} is greater than Φ_1^{Cl}, in conformity with results reported by Ussing (1949 b).

During one period (see Fig. 6) the potential-controlling circuit was used to keep φ at zero. During this period Φ_{a1}^{Na} increased markedly (as was to be expected, cf. 2.3.D.a). Φ_1^{Cl} could be expected to decrease during this period, but in fact it remained approximately constant. Consequently, quite improbable values of the partial conductances were obtained, being far greater than the total electrically measured conductance. The experimental period was short, however, and the result seems to indicate that no steady state was reached. As high specific activities of the isotopes are required for such experiments, it has not yet been possible to repeat this experiment more properly. The behaviour of the Na flux however, seems to agree with the views presented in this paper.

c. ***The active transport of salt.*** The flux of salt, Φ^{NaCl} calculated from the changes in the concentration of the solution in 1 with formula (2.2.1), agrees fairly well with Φ^{NaCl} calculated according to eqn. (1.5.5.), as may be seen from Fig. 6. Other similar experiments are given in connection with the study of the action of diuretics and CO_2 (Figures 12, 16 and 18, see also Table 9). The Φ^{NaCl} values in the figures and in Table 9, calculated according to (2.2.1), were not corrected for a net flux of water, and should be about 10 $\mu\mu$mol cm^{-2} sec^{-1} larger (cf. 2.2.f). For a discussion of this correction see p. 87.

D. Comments on the results obtained for isolated frog skin.

The validity of the assumptions made for the treatment of the experimental data will first be discussed. In order to make possible a direct application of the formulas derived in the theoretical part, it was assumed that the skin behaved as a homogenous uncharged membrane, in which the ions diffuse as in free solution. This assumption needs further consideration which will also involve the nature of the possible sodium carrier complex and the character of the sodium pump.

The experiments show that the skin does not behave as the simple membrane of the assumptions. For this simple membrane, the formulas require a G_a^{Na}/G^{Cl} ratio equal to the ratio of their mobilities $u^{Na}/u^{Cl} = 0.67$. The experiments give a G_a^{Na}/G^{Cl} ratio of 1.5 for the skins with solutions of the same concentrations on both sides and 2.5—3 in the skins with a 7 mM solution on the outside (Table 6 and 8). Such ratios could be explained by assuming a much higher mobility for sodium ions in the skin than in water. But they may also be explained (cf. TEORELL 1951) by assuming fixed ions in the membrane which virtually change the mobility of the diffusible ions.

The probable occurrence of carrier molecules in the skin makes it likely that some charged molecules occur, though they may be neutralized by other charged molecules (proteins) to give an approximately uncharged membrane. In any case, it must be assumed that the total concentration of the Na ions (free or bound) in the membrane is higher than if no Na carrier

were present, and the presence of a carrier invalidates the assumption that all Na ions diffuse as in free solution. Before we carry the discussion further, let us consider the nature of the »sodium pump».

a. ***Some remarks on the nature of the »sodium pump» of the frog skin.*** In order to explain the fact that the transport of Na ions results in a transfer of electricity equivalent in amount to the number of Na ions transfered in the short-circuited skin, it must be assumed that the carrier, after delivering the Na ion at one side of the membrane, returns to the other side without taking a positive ion on its way back in place of the Na ion. This seems to require a change in the carrier at the boundaries of the membrane.

Ussing (1949 b), in his hypothetical sodium pump, assumes a negatively charged carrier molecule in the active membrane which he — on good grounds, it seems — identifies with the part of the cell membrane of the basal epithelial cells which is directed inward to the chorium. A substance, acetylcholine, is synthetized in the cells on one side of the membrane (1) and diffuses through the lipoid membrane to the cells on the other side (2). Here the acetylcholine is split by an enzymatic reaction into choline ions and acetate ions. The latter ions diffuse back owing to the concentration gradient across the membrane and are replaced by Cl ions diffusing in the opposite direction to maintain electroneutrality. The sodium and choline ions are impermeable and can cross the membrane only by exchange diffusion via the carrier.

With this hypothetical transport system it is difficult to explain the current produced by the skin as well as changes in the net transport of sodium with changes in the electrical potential across the skin. The system would also require a rather marked exchange diffusion of Na ions, which is contrary to the results obtained in this investigation.

A sodium pump hypothesis which offers a better explanation of the experimental observations is that proposed by Conway (1951). Conway's sodium pump will therefore be described in some detail. Conway assumes some different metal catalyst systems (which may be thought of as cytochromes), one of which is the carrier for the cation (Na^+). When this catalyst is reduced by a catalyst of the flavoprotein type in the cell it may be assumed to expose a free or extra negative charge, and upon this Na^+ is supposed to be more or less specifically adsorbed. The other metal catalysts take part in the transit of electrons, which is supposed to be bound to occur along a certain route through the cell membrane and back to some final acceptor in the cell. One

of these other catalysts oxidizes the reduced carrier catalyst in the outer (cell) membrane region, and the Na ion is liberated. The oxidized catalyst is then supposed to return to the first side, perhaps by molecular rotation. Anions are removed from within the cell by the potential difference established by the active carriage of Na ions. In this system, differences in the oxidation-reduction potentials are assumed to be the driving force. The electrons liberated at the oxidation of the metabolites take with them the sodium ions out of the cell on their way (determined by the spatial arrangement of the catalyst systems) to the final acceptor (oxygen).

This hypothesis has the advantage that it readily explains the current produced by the skin, especially the direct relation between the net transport of sodium ions and the equivalent amount of current produced in the short-circuited skin, the relation found in experiments described by USSING and ZERAHN (1951) and in this investigation. It also explains the dependence of the electrical potential on the oxygen supply as well as the inhibition of the potential by several respiratory inhibitors (see 4.1).

If special consideration is given to the sodium ions and the sodium carrier, CONWAY's hypothesis may be taken to imply schematically the following course of events. At one boundary of the membrane there is a continuous supply of sodium carrier molecules, and at the other these sodium carrier molecules disappear. This means that the Na ions are subject to a continuous drag which tends to force them from, say, boundary 1 to boundary 2 of the membrane. The magnitude of this force, which may be identified with the force exerted on the ions due to the active transport potential as defined in the theoretical part, depends on the difference in the oxidation-reduction potential of the catalyst systems engaged (cf. CONWAY 1951). It may be assumed to be fairly constant in the steady state with a sufficient supply of oxygen and fuel. However, this does not rule out the possibility that changes in the electrical potential difference across the skin may affect the redox systems. The results obtained seem to indicate that the active transport potential is changed by variations in the electrical potential across the skin. (Tables 5 and 7).

The sodium carrier complex may be assumed to be more or less dissociated, so that an electrical field or a chemical potential gradient may be expected to tend to move Na

ions in the carrier system along the gradient. It may even be assumed that the carrier complex is undissociated inside the (lipoid) membrane, more or less like a crystal, but dissociates at the boundaries. In both cases the carrier complex may be assumed to contribute to the conductance of the Na ions through the membrane.

In this connection, the effects of an electrochemical gradient across the skin on the transport of Na ions by the carrier will be considered. It will be seen from Fig. 4 that when $c_1 = c_2$ and φ is changed from its resting value in the open-circuited skin to zero, Φ_{a1}^{Na} increases markedly. A similar result is demonstrated by Fig. 6 when $c_1 \approx 7$ mM. Evidently a low potential in 2 will make it easier for the Na ions to diffuse out into the solution in 2 while a high potential there has the reverse effect. A sufficiently high electrical or chemical potential gradient may even be expected to run the net sodium transfer backwards in the carrier system, i. e. from 2 to 1 or against the active transport potential.

It may be assumed that there are other paths besides the carrier system for the Na ions to diffuse through the membrane — maybe the same as for Cl and other passive ions. The proportion of the Na ions which pass this way may be quite small, as it is possible to imagine a membrane with pores fine enough to let through Cl and K ions while the Na ions are essentially impermeable (cf. CONWAY 1947). On the other hand, it is quite possible that the sodium permeability is as large compared with that of Cl as might be expected from the difference in the mobility of the ions in water.

b. ***Frog skin as a charged membrane.*** It is difficult to regard a specific carrier in the same way as a membrane charge in the sense used by Teorell-Meyer-Sievers, cf. also TEORELL (1951). This theory demands that the concentration of both positive and negative free diffusible ions should be equally affected by the fixed ions in the membrane, and it does not account for undissociated complexes. In a later paper (TEORELL 1952), a specificity of the fixed ions to different diffusible ions

is taken into consideration, though the theory is not complete in this case.

The idea that frog skin behaves as a charged membrane as in the theory of Teorell-Meyer-Sievers has been tested, apparently with good success, and we will therefore briefly consider these investigations. HASHIDA (1922) observed that with the inside of the skin bathed with 120 mM NaCl solution, frog skin when treated with different dilutions of NaCl solutions on the epithelial side showed a characteristic potential-log (concentration) curve with a maximum φ at a c_1 corresponding to about 20—30 mM NaCl. With lower concentrations φ first decreased and later changed its sign for c_1 below 1 mM NaCl. Similar φ curves were obtained for other sodium (and lithium) salts, while salts with cations like Ca^{++}, K^+ and NH_4 usually gave a resting potential about zero with the same concentration on both sides, and dilutions of these salts on the outside influenced φ much less than was the case with sodium salts.

Similar results were obtained by GREVEN (1941). He found that the characteristic φ-log (c_1) curve could be explained by the Teorell-Meyer-Sievers theory, and he used his data to calculate the membrane charge of the skin.

Later, MEYER and BERNFELDT (1946) used potential measurements to estimate the charge of the frog skin membrane and arrived at results similar to those of GREVEN. With KCl they found that the skin charge even changed sign.

The interpretations of GREVEN and MEYER *et al.* are open to criticism. They have not taken into account the possible changes in the spontaneous potential of the frog skin for the wide variations in the concentration of the outside solution. Nor is it possible from their point of view to explain why the skin behaves so differently to salts of other cations than Na^+ (and Li^+). It seems in fact rather improbable that the charge of the membrane should suddenly disappear when the skin is exposed to these other ions. The rather specific behaviour of frog skin in cases when sodium salts are used seems to suggest, in the light of later investigations, that the results have something to do with the active transport of Na ions.

c. ***The concept of a specific carrier used in a hypothesis to explain the characteristic "potential-log(concentration) curve„ of frog skin.*** The sodium carrier discussed above will now be used in an attempt to explain the characteristic φ-log (c_1) curve described. It will be found that it not only gives a satisfactory explanation of the behaviour of the skin in sodium salt solutions, but that it can also explain the small effect on φ of the other salt solutions mentioned.

The hypothesis is as follows. The frog skin membrane is supposed to be inhomogeneous, in so far as there are some parts of the membrane where active transport does not take place but where both Na and other ions diffuse through the skin as passive ions, maybe through fine pores. If these parts are assumed to be a continuous water phase, they must constitute only a small fraction of the membrane in order to explain the low permeability of the skin. The other part of the membrane contains a sodium carrier, and here the active transport takes place. It may be thought of as a lipoid membrane, essentially impermeable to other ions than those transported by the carrier. For the general concept of the structure of cell membrane see for instance DAVSON & DANIELLI (1943). The carrier complex may be little dissociated in the membrane, but is assumed to dissociate to a certain extent at the boundaries of the membrane.

We assume that the partial conductances of the passive ions, mainly G_p^{Na} and G^{Cl}, can expressed by eqn. (1.3.12) and that $G_a^{Na} = (F^2/\delta) u^{Na} X$, is the contribution of the sodium complex to the partial conductance of Na ions. Here X is defined as the »*effective sodium carrier concentration*» in the membrane and is assumed to be independent of c_1. X may be quite low if the mobility of Na ions is assumed to be large in the carrier system (as seems quite probable). However, for the convenience of calculation u^{Na} is assumed to be the same as in water.

We further assume that in the part of the membrane where active transport of sodium takes place the active transport potential φ_a^{Na} has a constant value independent of c_1 and the electrical potential across the membrane. The effective active transport potential of the whole membrane, φ_a^{Na} is then related

to the transport potential in the part of the membrane where active transport takes place, φ_a^{Na}, by an expression like (1.6.3). Using experimentally determined values for φ_a^{Na} and X, it is possible to obtain φ for different c_1 by means of eqn. (1.5.3).

When the G_a^{Na} G^{Cl} ratio is known, it is possible to determine X from above-mentioned expressions for the partial conductances. This has been done for the case when $c_1 = c_2$, i. e. Ringer's solution on both sides, and also in the experiments when c_1 was about 7 mM, using the G_a^{Na}/G^{Cl} ratio in Tables 6 and 8. When $c_1 = c_2$, X becomes 153 (62—244) mM, corresponding to a G_a^{Na} G^{Cl} ratio of 1.5 (1—2), and in the experiments when c_1 is about 7 mM, X is 104 (70—177) mM, corresponding to a G_a^{Na} G^{Cl} ratio of 2.5 (1.9—3.8). All calculations assume $\alpha = 1$ in eqn. (1.3.12).

In order to illustrate the hypothesis, calculations were made for a hypothetical skin with an »effective carrier concentration» X of 100 mM and with NaCl solutions on both sides. c_2 was assumed to be constant (= 120 mM) while c_1 varied. The activity coefficients of the ions were calculated according to eqn. (1.3.8). φ_a^{Na} was assumed to be 70 mV when $c_1 = c_2$. This seems to be a reasonable value, in view of the experimental results.

The results of the calculations are given in Fig. 7, which also gives the total conductance ($G = G_a^{Na} + G_p^{Na} + G^{Cl}$) for the different c_1 values.

It is interesting to compare the general features of the behaviour of the hypothetical skin with the experimental results. As seen from the figure, the φ_a^{Na} value increases with decreasing c_1. This is in agreement with the findings, cf. Tables 7 and 9. Unfortunately, the Ringer's solution used in the experiments with $c_1 = c_2$ and that used in those with $c_1 < c_2$ had different pH values so the results are not quite comparable.

The form of the φ curve agrees with that of similar curves of HASHIDA (1922) and GREVEN (1942). Experiments of the same kind as those reported by HASHIDA and GREVEN have been performed in this investigation. At the same time as φ was measured, the G of the skin was recorded. The results of two such experiments are given in Fig. 8, one for a skin with

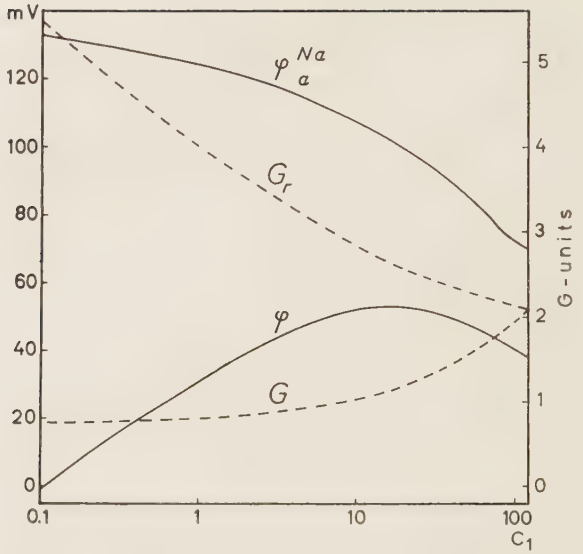

Fig. 7. The φ-log (c_1) curve of a hypotetical frog skin with an »effective carrier charge», X, for sodium ions of 100 mM and an active transport potential φ_a^{Na} of 70 mV when $c_1 = c_2$. The total conductance G and the function G_γ are also given. For further explanation see text, 2.3.D.c.

a high conductance and the other for a skin with low conductance.[1] The curves are typical for such experiments and it will be seen that the form of the φ curve agrees fairly well with that of the hypothetical skin.

The form of the G curve for the hypothetical skin does not agree so well with that of the experimentally determined one. This becomes evident if the G-values of the two curves are multiplied by $\{c_2 \ln (a_2/a_1)\} : \{c_2 - c_1) - \psi\}$ — which expression may be arrived at with the aid of eqn. (1.3.12) in which case a function called G_γ is obtained. This procedure

[1] It should be remarked that the same form is obtained for both the φ and G curves when a series of dilutions of Ringer's solution with isotonic sucrose solution are used instead of Ringer's diluted with distilled water. It therefore seems improbable that changes in the osmotic pressure of the outside solution is the main factor producing the change in the resistance of the frog skin when exposed to different concentrations of electrolyte solutions on the epithelial side, as suggested by LÖFGREN (1951).

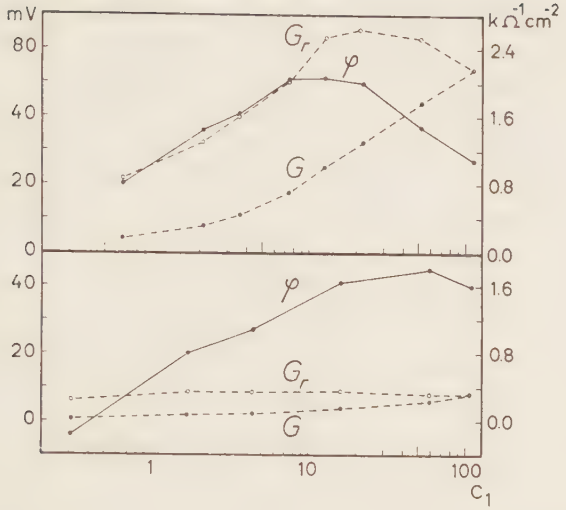

Fig. 8. Typical φ-log (c_1) curves and G values from two experiments, In the upper part of the figure are shown the results from a skin of high conductance in Ringer's solution with bicarbonate (pH 8.2), and in the lower part the results from a low conductance skin in Ringer's solution with phosphate buffer (pH 7.4). The G_r function is also given for comparison with the theoretical skin in Fig. 7.

is similar to that adopted in an earlier paper (LINDERHOLM 1950), though in that paper another (empirical) expression was used.

The less satisfactory agreement of the G curves seems to indicate that the assumption of linear concentration gradients through the membrane in the hypothetical skin is too rough an approximation. The form of the concentration gradient may, for instance, depend on that of the gradient of the active transport potential through the membrane. It may also mean that X is not constant. The assumption that X is constant is certainly open to criticism, as well as that of a constant φ_a^{Na}. However, so many of the features of the hypothetical skin agree with experimental results that the hypothesis seems justified, especially as it is possible to estimate from the calculated X values changes in the carrier system induced by various factors that may affect the skin. In addition, it is possible to explain a small effect on the φ-log(c_1) curve of

the skin when KCl or, $CaCl_2$ solutions are used, assuming an »effective carrier concentration» approaching zero.

X is in itself only a relative measure of the state of the carrier system or changes in it, while G_a^{Na} is an absolute one and offers better possibilities of comparing various factors that may affect the carrier. G_a^{Na} may be calculated as $G_a^{Na} = G - G^{Cl} - G_p^{Na}$, where $G_p^{Na} = (u^{Na}/u^{Cl})\, G^{Cl}$. The mean G_a^{Na} value calculated in this way was 0.25 $k\Omega^{-1}$ cm^{-2}, both for 13 open-circuited skins and for 10 short-circuited skins with $c_1 = c_2$. The mean G_a^{Na} value from the periods before the introduction of drugs or other changes was 0.13 $k\Omega^{-1}$ cm^{-2} in experiments 5.1, 5.2, 6.1 and 6.2, where c_1 was 7—8 mM. There is evidently a tendency for G to decrease with solutions of low concentration on the outside.

The G_a^{Na} values estimated in this way make it possible to evaluate the actual transport potential of the »sodium pump», φ_a^{Na}. Substituting the values of G_a^{Na} and G_p^{Na} in eqn. (1.6.3), φ_a^{Na} is found to be 150—170 mV in open-circuited skins both when $c_1 = c_2$ and when $c_1 = 7$—8 mM. In view of the difference in the oxidation-reduction potential between flavine and cytochrome enzymes (cf. GODDARD 1945), this value of φ_a^{Na} seems plausible for a redox pump of the type proposed by CONWAY.

This discussion leads us to the conclusion that it is not necessary to assume that frog skin is a charged membrane as has been supposed by GREVEN, and MEYER and BERNFELDT. There are, on the contrary, *good reason to believe that the concentration of fixed ions in the membrane in the sense of the Teorell-Meyer-Sievers theory is small in frog skin, and may be disregarded compared with the concentrations of ions in the solutions used in the experiments with radioactive isotopes. The proposed structure of the frog skin membrane will therefore not invalidate the formulas used.*

d. ***Evidence supporting the assumption that*** Cl^- ***(and*** Br^-***) behaves as a passive ion in isolated frog skin, and the importance of the active transport potential for the calculation of the partial conductance.*** The experiments described above (2.3.B) provide good evidence that Cl^- behaves as a passive

ion. Within the limits of experimental error, the Cl ions seem to follow the conditions imposed by eqn. (1.2.1). The following considerations will show how important it is for the calculation of the partial conductance of an ion that the assumption about whether the ion is passively or actively transported is correct. On the other hand, it is possible to draw indirect conclusions from calculated values of the partial conductance whether an ion is actively transported or diffuses passively (cf. 1.4).

In fact, the good agreement between $G_a^{Na} + G^{Cl}$ and G measured electrically seems to be a good justification of the assumption that the Cl ion is passively and the Na ion actively transported. To make this clear, we will first consider the experiments in which the concentration was the same in the solutions on both sides of the skin, and assume that Na^+ is a passive ion.

If G^{Na} is calculated from eqn. (1.3.14), thus disregarding an active transport potential, the G^{Na} values obtained differ markedly according to whether Φ_1^{Na} or Φ_2^{Na} are used, i.e. the flux in the direction of the active transport potential or in the opposite direction. With Φ_1^{Na} the G^{Na}-values become greater than the electrically measured G. The quotient G^{Na}/G 1.26 (calculated from experiments on 4 frog skins and corresponding to measurements during 36 periods of 15—30 minutes). With Φ_2^{Na} the G^{Na}-values become small, and the corresponding quotient G^{Na}/G 0.22 (calculated from experiments on 3 frog skins and corresponding to measurements during 17 periods of 15—30 minutes).

If we further assume that Cl^- is the actively transported ion in the direction 2 to 1, which would also give a potential and a current in the same direction as an active transport of sodium in the direction 1 to 2, even more improbable values were obtained. With this assumption, if G_a^{Cl} is calculated from the flux of the Cl ions from 1 to 2, it is greater than the value obtained in the calculations when Cl is assumed to be a passive ion, and the sum of G^{Na} and G_a^{Cl} is even greater as compared with G than in the case when both Na and Cl are assumed to be passive ions.

If the partial conductances are calculated from the fluxes in the direction from 2 to 1, the G_a^{Cl} values will be smaller than if Cl ions are regarded as passive, and, combined with the low G^{Na} values obtained with the assumption of Na^+ as a passive ion, the sum of G^{Na} and G_a^{Cl} will form only a small fraction of G.

Any condition between these two extremes — i. e. a partial active transport of Cl ions from 2 to 1 and a partial active transport of Na ions from 1 to 2 — would tend to give too high values for the sum of the partial conductances as compared with the electrically measured G when the partial conductances are calculated from the inward fluxes of Na and Cl ions, and too low values when calculated from the corresponding outward fluxes.

In the experiments with solutions of different concentrations on each side of the skin, the effects mentioned above will be still greater, owing to the greater chemical, electrical (and active transport) potential differences across the skin.

If Na^+ is here assumed to be a passive ion, the partial conductance obtained from eqn. (1.3.14) gives high values when calculated from Φ_1^{Na}, the G^{Na}/G quotient being in the order of 10 to 25.

If the reasoning is carried further, analogously to that above, and Cl ions are taken to be actively transported, still more improbable values will be obtained.

e. ***The importance of small amounts of other ions than*** Na^- ***and*** Cl^- ***for the validity of the formulas used.*** The Ringer's solutions used contain small amounts of K, Ca, and bicarbonate or phosphate ions. Since they occur in low concentrations and as their mobilities in the skin may not differ very markedly from those of Na and Cl ions it may be assumed that the flux formulas, which are strictly valid only in the single salt case, are good approximations. The same should then be true for the calculated value of the partial conductance of the passive Cl ions, G^{Cl}. (For further discussion of possible errors see below 2.3.D.g, p. 89.)

The high content of K and other ions within the skin (RUBIN 1936) may seem to invalidate the »single salt assumption». These ions, however, do not take part in the diffusion processes, and may be regarded as located to a great extent in, for instance, connective tissue cells. The cells which take part in the active transport of Na ions are probably quite permeable to this ion, and may have a low K ion concentration. CONWAY, FITZGERALD and MACDOUGALD (1945) have made such assumptions about frog kidney tubule cells, which were supposed to transport Na ions.

If ions leak out from the skin, as may occur, for instance, when i is suddenly changed from the resting value in the open-circuited skin to zero, or when drugs are added (see Chap. 3), the assumptions are no longer completely valid. But this leakage of ions may usually be regarded as small.

Another possibility is that ions are continuously produced in the skin and continuously leave it. We may think of the production of carbonic acid. It is proposed by HUF, PARRISH and WEATHERFORD (1951) that this may account for the usually slightly smaller net uptake of Cl ions than of Na ions by the skin. The reasoning below will take such effects into consideration, however, and the errors due to leakage of ions out from the skin may be regarded as small compared with other experimental errors.

Error in φ_a^{Na}. φ_a^{Na} or ξ_a^{Na} is a factor in eqn. (1.4.3) which is used for the calculation of G_a^{Na}. In order to estimate the possible effect of the small amounts of other ions on G_a^{Na}, their effects on i_a^{Na} will first be considered. As described above (p. 30, 31) two methods for the calculation of i_a^{Na} may be used. It can be shown that, at least when $c_1 = c_2$, φ_{aI}^{Na} will be too low if other ions are present, and the same can be said about b_{aII}^{Na}, except in one case. If $c_1 = c_2$ and $\varphi = 0$ the value of φ_{aII}^{Na} is independent of the presence of passive ions, as may be seen directly from eqn. (1.7.3). In such experiments, the $\varphi_{aI}^{Na}/\varphi_{aII}^{Na}$ quotient is 1.3 (see 2.3.B.e, p. 67). In this case, however, the numerical evaluation of φ_{aII}^{Na} is such that even a small error in one of the experimental quantities may give an entirely erroneous result, and it therefore seems that the error due to the

occurrence of ions other than Na^+ and Cl^- is small as compared with experimental errors from other sources.

Error in the G values. The effect of a too small q_a^{Na} on the calculation of G_a^{Na} has been touched upon earlier (p. 83). When G_a^{Na} is calculated according to eqn. (1.4.3), it can be shown that a too low q_a^{Na} will give high G_a^{Na} values when Φ_{a1}^{Na} is used and the contrary when Φ_{a2}^{Na} is used. As may be seen from Table 6, it does not prove to be the case that the G_{aII}^{Na} values calculated from the Φ_{a1}^{Na} are high and the G_{aII}^{Na} values calculated from Φ_{a2}^{Na} low. To judge by the G_{aII}^{Na}/G^{Cl} ratio, the contrary seems to be true. In this case also experimental errors other than those due to the small amounts of other ions than Na^+ and Cl^- seem to be of greater importance.

The best evidence that the small amounts of other ions have little effect on the calculations seems to be the good agreement between the electrically measured G and the sum of G_{aII}^{Na} and G^{Cl} calculated from the flux measurement. If we first consider the experiments in open-circuited skins, it can be seen from the $G/(G_a^{Na} + G^{Cl})$ ratio in Table 6 that this sum seems to be a little smaller than the electrically measured G. This might be expected, since the other ions should contribute to the conductance of the skin. Other ions than Na^+ and Cl^- contribute about 7 per cent of the total conductance of the phosphate Ringer.

In the short-circuited skin, the $G/(G_a^{Na} + G^{Cl})$ ratio (Table 6) seems to show that the sum of the partial conductances exceeds the electrically measured conductance. As the skins were not so stable and there was often a slight continuous change in the conductance or the current through the skins in these experiments, these values are probably less accurate than those obtained from open-circuited skins.

In the skins with $c_1 \approx 7$ mM, there is also a good agreement between $G_a^{Na} + G^{Cl}$ and the electrical G (Table 8). Other ions than Na^+ and Cl^- contribute about 3 per cent of the total conductance of the bicarbonate Ringer. *Taken together, the results seem to indicate that the small amount of other ions than Na^+ and Cl^- in the solutions can be regarded as intro-*

ducing only minor errors into the calculation of the partial conductances.

Errors in Φ^{NaCl}. We will finally consider the effect that the presence of small amounts of other ions might be expected to have on the calculated Φ^{NaCl} values. G_{a1}^{Na} and η_{a1}^{Na} were usually used in eqn. (1.5.5), and an error in the latter of these quantities is of the greatest importance. The presence of other ions will as a rule decrease this value, and we may therefore expect a Φ^{NaCl} value lower than if no other ions were present.

In the experiments with $c_1 \approx 7$ mM, the Φ^{NaCl} values obtained from eqn. (1.5.5) (see Table 9) seem to be slightly lower than the values obtained from (2.2.1), especially if the latter are corrected for an assumed net water flux of 5 μl cm^{-2} hr^{-1}. The mean difference between the two methods of calculating the Φ^{NaCl} would then be about 13 $\mu\mu$mol cm^{-2} sec^{-1}, and part of this difference may be due to the presence of small amounts of other ions (cf. also below, 2.3.D.f). Due to the great difference in electrochemical potential between the passive positive ions in the solutions 1 and 2, these ions will be of greater importance in the experiments where $c_1 < c_2$ than in those with $c_1 = c_2$. In any case, the values obtained seem to indicate that (1.5.5) is a fairly good approximation.

It can be shown quite generally that in the case when one ion of a salt is actively transported through a membrane, the presence of other electrolytes with different anions and cations will decrease the transport of this particular salt. Part of the energy of the active transport will be used for the transfer of the ions of these other electrolytes.

f. ***Changes in the composition of the bathing fluids as a result of the active transport.*** The interesting investigations carried out by Huf and collaborators (1951) seem to indicate that K^+ and Ca^{++} behave as passive ions. When Na and Cl ions accumulate on the inside of the skin, K and Ca ions accumulate on the outside. This is to be expected if they behave as passive ions with regard to the electrochemical potential difference of the ions in the two compartments. Katzin (1940) used K^{42} and found with chemically similar solutions on both

sides of the skin a net outward flux of K ions, $-\Phi^{K}$, of 5.5 –16.7 $\mu\mu$mol cm^{-2} sec^{-1} for lower ranges of potassium concentration in the bathing solutions. At the same time there was a net inward flux of Na ions. His results favour the view that K^+ is a passive ion in the skin. KATZIN (1939) also measured the outward total flux of radioactive rubidium, $-\Phi_2^{Rb}$, in frog skin bathed with distilled water on the outside, and found it to be 125 $\mu\mu$mol cm^{-2} sec^{-1} when the inside of the skin was bathed with isotonic RbCl solution.

We will now use the values of G^{Cl} obtained in this investigation to calculate the probable flux of K ions in the experiments, and to make some comparisons with the above mentioned results. We assume that K ions have the same mobility in the membrane as Cl ions. The concentration of the K ion in the Ringer's solutions used is about 3 per cent of the concentration of the Cl ion, and we therefore assume the G^{K} to be about 3 per cent of the G^{Cl}. It is then possible to calculate Φ^{K} approximately by eqn. (1.3.13). For a frog skin with φ = 40 mV and $G^{Cl} = 0.4$ kΩ^{-1} cm^{-2} (Table 6) and $c_1 = c_2$, we find $-\Phi^{K} = 5$ $\mu\mu$mol cm^{-2} sec^{-1}. This figure is of the same order of magnitude as that given by KATZIN (1940), but as he does not give the exact concentration of K ions in his solutions further comparisons are impossible. Ca ions may be assumed to have lower mobilities in the skin than K ions have, and the net flux of Ca ions should therefore be lower than that of K ions.

The calculated flux values for K (and Ca) ions agree with results reported by HUF and WILLS (1951). They state that for each 100 μEq. Na^+ which are removed from the fluid at the epithelial layer only 4 to 1 μEq. K^+, Rb^+ and Mg^{++}, or even less, are given off from this layer. As Φ^{Na} in the frog skins investigated here is usually a little less than 200 $\mu\mu$mol cm^{-2} sec^{-1}, the proportion of Φ^{Na} to the calculated Φ^{K} is the same as that given by HUF *et al.*

The flux values of K and Ca ions are far too low to give any appreciable change in the composition of the bathing solutions in the experiments where $c_1 = c_2$.

If the probable flux of K ions is calculated in a skin with $c_1 \approx 7$ mM, $\varphi = 54$ mV, $G^{Cl} = 0.073$ kΩ^{-1} cm^{-2}, (Tables 8 and 9) $-\Phi^{K}$ is found to be 2 $\mu\mu$mol cm^{-2} sec^{-1}.

This figure may be compared with the value of the outward flux of rubidium in the above mentioned experiment described by KATZIN. If the Rb concentration were 3 mM instead of 120, a $-\Phi^{Rb}$ of $125 \times 3\ 120 - 3$ $\mu\mu$mol cm^{-2} sec^{-1} of rubidium would be expected. As the total inward flux in this experiment is probably so small that it can be neglected, the total outward flux, $-\Phi_2^{Rb}$, is approximately equal to $-\Phi^{Rb}$, and is of the same order of magnitude as the calculated Φ^K.

Even with a $-\Phi^K$ of 5 $\mu\mu$mol cm^{-2} sec^{-1}, the change in the composition of the outside solution in the experiments when $c_1 \approx 7$ mM is small. The volume of the outside solution is about 8 ml, and the increase in the K ion content during a 6 hour period is only about 1 per cent of the total electrolyte content. This is of importance for the estimation of the NaCl concentration in 1 from the conductance measurements (cf. 2.3.f). The negative ions, Cl, HCO_3, and phosphate ions, may all be assumed to follow the electrochemical potential gradient, and the net fluxes of these ions in the experiments considered are probably rather small, as the electrical and chemical potentials balance each other well. The total potential difference for the Na ions is also low and changes in the concentration of this ion may be expected to be small.

g. ***Some possible factors which may invalidate a comparison between the electrically measured conductance of a membrane and the sum of the partial conductances obtained from measurements of ionic fluxes by means of tracers.*** Three such factors of possible importance will be discussed.

Convection-free layers. If convection-free or »unstirred layers» occur at a membrane they will tend to decrease the flux of an isotopic ion through the membrane, since part of the driving force is expended by the diffusion process through the unstirred layers. Consequently, a lower partial conductance will be obtained by calculation with the formulas in Chapter 1 than if no unstirred layers were present. At the same time however, the electrically measured conductance is independent of the unstirred layer if this has the same conductance as the surrounding stirred solutions.

In frog skin, it seems quite possible that the main diffusion barrier and the main electrical resistance are due to one or a few very thin membranes, while in the other layers of the skin diffusion may occur almost as easily as in water. The chorium layer of the skin, for instance, like all connective tissue, offers a resistance to ions which does not differ much from that of water. As, however, diffusion in water is a slow process, even such a thin »unstirred» layer may be a considerable diffusion barrier.

In order to obtain an approximate idea of the possible effects of convection-free layers in the frog skin, it may be regarded schematically as consisting of a very thin membrane, representing the main diffusion resistance of the skin, and an »unstirred» layer of the same thickness as a frog skin, in which ions diffuse as in water. TAYLOR (1935) found frog skins to be 0.3 mm thick and similar values, 0.2—0.3 mm, have been estimated in the present investigation from the weight of pieces of skin with known area, assuming the specific weight to be 1.

It is probable that the electrical potential gradient in the »unstirred» layer is negligible. Let us consider a skin with Ringer's on both sides, assuming a net flux of Na ions of say 500 $\mu\mu$mol cm^{-2} sec^{-1}, which is higher than the ionic fluxes recorded in the present experiments. Eqn. (1.3.13) can then be used to estimate the ratio between the concentrations at the two borders of the unstirred layer. If the specific conductance of this layer is the same as in Ringer's solution, about 10 k Ω^{-1} cm^{-1}, and the specific partial conductance of Na ions is about 3.3 k Ω^{-1} cm^{-1}, the concentration ratio which corresponds to the flux mentioned is about 1.02. The retardation of a flux of the assumed magnitude by the »unstirred» layer is thus so small that it can probably be neglected. The same result is obtained when the »unstirred» layer adjoins a solution of concentration 7 mM and a flux of 50 $\mu\mu$mol cm^{-2} sec^{-1} is assumed (cf. Table 9). To put it in another way, it may be said that in the experiments reported only a small fraction of the total potential available for the transport of the ion is used up in transport across convection free layers.

Local circuits. Local circuits may occur in the skin due to the fact that in some parts of it active transport takes place while in other parts there is mainly passive diffusion. As the distances between such different sections of the skin are probably small the potential differences between the surfaces of such different sections cannot be significant. If they were, they would undoubtedly affect the flux values. In view of the current densities, 1 mV is probably a high figure for such potential differences.

In order to estimate the change of flux for a small change in the electrical potential, an expression was used of the form $\Delta\Phi_1' \Delta\eta = \Phi_1' f'(\eta)$, which was obtained from the flux formula for the case when $c_1 = c_2$. It was then found that a change in the potential of 1 mV in the range $\pm$ 100 mV would change the total flux of an ion by 1—3 per cent.

These figures are also of importance for the estimation of the error that might have arisen in the experiments when the potential-controlling circuit was used. The potential may then have deviated slightly from the ideal zero value. A deviation of 1 mV (and greater deviations rarely occurred) would evidently not affect the flux values much.

Carrier exchange diffusion. If carrier exchange diffusion takes place, the sum of the partial conductances calculated according to eqns. (1.3.14) and (1.4.3) with correct assumptions about the actively and passively transported ions should be greater than the total electrically measured conductance (cf. 1.3). The good agreement between the electrically measured G and the sum of $G_{\prime\prime}^{\mathrm{Na}}$ and G^{Cl} seems to be good evidence that such exchange diffusion does not occur to any significant extent.

h. ***Net transport of salt.*** The net transport of salt Φ^{NaCl}, calculated according to eqn. (1.5.5) for the experiments with Ringer's solution on either side of the skin agrees with the Φ^{NaCl} through the skin under similar conditions as determined by other methods. Huf, Parrish and Weatherford (1951) report some experiments which are comparable to those in the present investigation. They used the skin bag method, described

by HUF (1935), and determined the concentration of the ions chemically. In three experiments in which they used Ringer's solution of physiological strength and pH 7.3 on the two sides of the skin their data allow a calculation of $\Phi^{Cl} = 144—205$ $\mu\mu$mol cm^{-2} sec^{-1} and Φ^{Na} of 180—222 $\mu\mu$mol cm^{-2} sec^{-1}. These values are well within the range of flux values obtained in this investigation. As mentioned above in 2.3.B, the results given in Table 7 seem to indicate a slightly greater net flux of Cl than of Na ions. This is probably due to inequality of the skins, and also to the fact that too few experiments were done to give good values. In this case, the chemical methods used by HUF *et al.* seem more reliable.

In the experiments where a dilute solution was used in 1, there was an opportunity to determine Φ^{NaCl} by two independent methods. The results obtained from eqn. (1.5.5) agree satisfactorily with those calculated from the changes in the concentration in the solution in 1 according to eqn. (2.2.1). Compare also the discussion on p. 87.

These results, taken together with the results discussed earlier, seem to provide a considerable amount of evidence to support the possibility and approximate correctness of treating frog skin according to the concepts presented in the theoretical part. They are in conformity with the results reported by USSING and collaborators (cf. USSING 1951). *The active transport of salt, the electrical potential across the skin (with equal concentration on either side), and the ability of the skin to produce an electrical current may then all be regarded as phenomena directly dependent on the active transport of sodium ions through the skin.*

Chapter 3.

The effect of some diuretic drugs and changes in pH (induced by CO_2) on the active transport through frog skin.

3.1. Earlier experiments.

Although several studies have been made on the water and electrolyte balance of frogs (ADOLPH 1933, KROGH 1937, JÖRGENSEN 1947 a, 1950 a,b,c, ELIASSEN and JÖRGENSEN 1951), the effect of the common diuretic drugs such as xanthine derivatives or mercury compounds has not been much investigated, neither with intact frogs nor with isolated frog skin.

$HgCl_2$ seems to have in principle the same diuretic action as the organic mercury compounds (MELVILLE and STEHLE 1928, PITTS and SARTORIUS 1950). It is therefore of interest that HERMANN (1870) and later BACH and OEHLER (1880) had already noticed that $HgCl_2$ was very effective in completely inhibiting the ability of the skin to produce an electric current. The effect of $HgCl_2$ has also been studied by the present author (unpublished experiments) who found that with 0.05 mM $HgCl_2$ on the outside of the skin the potential decreased to zero within a few minutes, and that the d.c. resistance of the skin also decreased, usually after an initial increase. When applied to the inside, the effect was slower and a higher dose was required to get the same effect.

The effect on the potential and the permeability of frog skin produced by several pharmacological agents, especially narcotics (ALCOCK 1906, GERSTNER 1940, HOFMANN 1943 and others), has been studied a good deal. Xanthine derivatives, on the contrary, do not seem to have been systematically investigated. UHLENBRUCK (1924) reports that caffein increases the current production of the skin.

Since acidifying agents are used as diuretics and also to potentiate the action of mercurial diuretics, the effect of changes

in pH is of interest in connection with the diuretics used. It has alreaIy been studied by Ussing (1949 b), Ussing and Zerahn (1951) and Huf, Parrish and Weatherford (1951). All the results reported indicate that a low pH inhibits active sodium transport through the skin.

Mersalyl and some xanthine derivatives (caffein, aminophylline) were found to have a marked influence on the electrical resistance and potential of frog skin (Linderholm 1950 and unpublished experiments). Their effect on the active transport of sodium and the ability of the skin to transport salt is studied in some experiments which are reported below. The methods described in earlier sections were used. Some results obtained when a change in pH was induced by CO_2 will also be presented, to illustrate the wide applicability of these methods.

3.2. The effect of Mersalyl (Salyrgan)[1].

Earlier experiments showed that the skins were sensitive to mersalyl when the drug was added to the inside solution while much less so when it was added to the solution on the outside. In the experiments described here the dose was varied little. Mersalyl solution, usually 0.1 ml, was added to the inside solution to give a concentration of about 0.1 mM. This dose was known to produce an effect. It was not much higher than the threshold dose, i.e. the lowest dose that gave a response. As the mersalyl solution is alkaline tests were made to ensure that a possible change in pH when the drug was added was unimportant for its effect.

Since the skins often changed considerably, and often continuously, after the addition of the drug, and since the resistance of the skin sometimes became very low, the results obtained from these periods of drug action may be regarded as less accurate than those obtained from periods before the intro-

[1] Mersalyl was obtained as the pure acid by courtesy af AB Leo, Hälsingborg. It was dissolved in frog Ringer's solution by adding an adequate amount of NaOH.

duction of the drug. This is especially true with regard to the comparability of the electrical resistance and the partial conductances obtained from the flux measurements. (cf. 3.2.C). The experimental data were treated as in 2.3.B. Two main types of experiments were performed, reported below under A and B.

A. Experiments with Ringer's solutions of the same concentration on each side of the skin.

The results of some typical experiments are shown in Figs. 9, 10, 11. Usually it takes about 15 to 30 minutes after the addition of mersalyl before the drug begins to act.

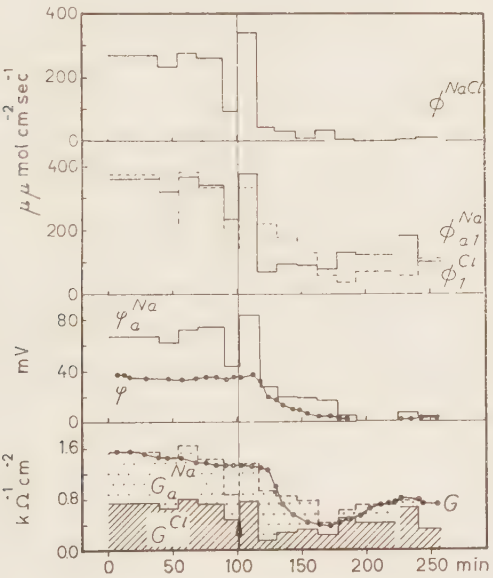

Fig. 9. Experiment 10.3 showing the effect of mersalyl on an open-circuited frog skin with Ringer's solution on either side. The drug was added to the inside solution as indicated by the arrow to obtain a concentration of 0.11 mM. The upper section of the figure gives the net transport of NaCl calculated by eqn. (1.5.5). The fluxes of Na and Cl ions were measured inward through the skin. For further explanation, see the text of Fig. 4.

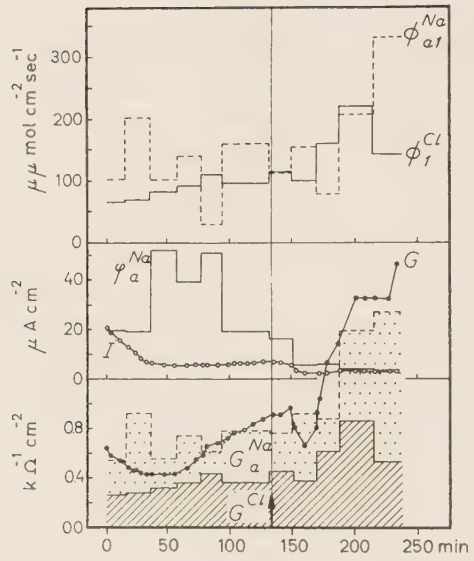

Fig. 10. Experiment 10.2. The effect of mersalyl in a short-circuited frog skin with Ringer's solution on either side. Mersalyl was added to the inside solution as indicated by the arrow, to give a concentration of 0.11 mM. The experiment is not quite typical as the current decreased considerably before the addition of the drug. The fluxes of Na and Cl ions were measured inward through the skin.

The essential effect of mersalyl is evidently a reduction in φ_a^{Na} and a marked decrease in Φ^{NaCl}. The G values, electrically measured, may increase or decrease (see the figures), though a decrease in G seems to be more usual during the first period of action.

Though it is not apparent from the figures referred to, the effect of the drug is at least partially reversible if Ringer's solution is substituted for the mersalyl containing solution on the inside of the skin. The results obtained will now be analyzed in some detail.

a. ***Experiments made to test whether the*** Cl ***ion behaves as a passive ion in frog skin under the influence of mersalyl.*** It is also important to known whether the Cl ion behaves as a passive ion in the experiments with mersalyl, when the formulas from the theoretical part are to be applied.

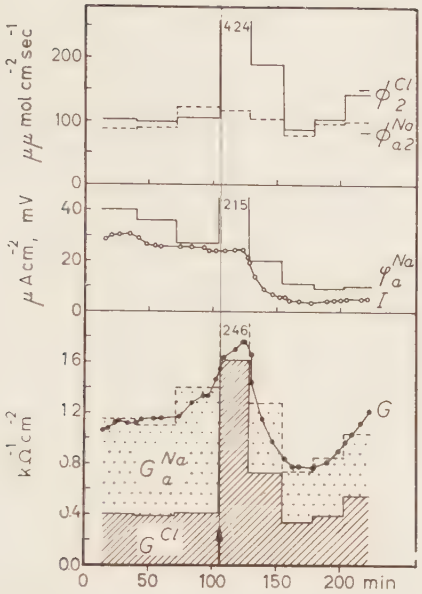

Fig. 11. Experiment 9.2. The effect of mersalyl in a similar experiment as that of Fig. 10. Fluxes of Na and Cl ions measured outwards through the skin. Mersalyl was added to the inside solution as indicated by the arrow, to give a concentration of 0.10 mM.

In some experiments in which φ was kept at zero, the flux of Cl ions was measured outwards and inwards for different skins. The results for some experimental periods in which the action of a 0.1 mM solution of mersalyl was evident are summarized in Table 10. Skins with approximately similar G values gave fairly similar values for the total flux of Cl ions. Though the experiments are few and the mean flux values uncertain, the results seem to indicate that the Cl ions behave approximately as passive ions and follow the requirements of eqn. (1.2.1), especially in comparison with the Na fluxes. In spite of the low active transport in these experiments, the Na flux values differ considerably in the two directions, and may account for the current produced.

b. ***Experiments with Cl^{36} and Br^{82} in combination to test whether these ions behave as passive ions during the action of mersalyl.*** In two experiments in which the frog skins were

Table 10.

Effect of mersalyl. Fluxes of Cl and Na ions inward and outward through different short-circuited skins ($q = 0$), the total conductance G, and the current through the skin I, with mersalyl (0.1 mM) present in the inside solution. Mean values from each experiment.

Exper. no.	n	Φ_1^{Cl}	G kΩ^{-1} cm^{-2}	Exper. no.	n	Φ_2^{Cl}	G kΩ^{-1} cm^{-2}	$\frac{-\Phi_2^{Cl}}{\Phi_1^{Cl}}$
10.2	4	157	1.4	9.2	4	132	1.0	0.8
11.2	4	116	1.3	15.2	3	147	1.1	1.3
Mean		137	1.4	Mean		139	1.0	1.1

Exper. no.	n	Φ_{a1}^{Na}	I/F	Exper. no.	n	$-\Phi_{a2}^{Na}$	I/F	$\frac{-\Phi_{a2}^{Na}}{\Phi_{a1}^{Na}}$
10.2	4	193	29	9.2	4	96	60	0.5

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}. I/F in $\mu\mu F$ cm^{-2} sec^{-1}.
n is the number of 15—30 minute periods included in each experiment.

exposed to the action of a 0.1 mM solution of mersalyl, the fluxes of Cl^{36} and Br^{82} were measured in the same direction simultaneously. The Br flux values were calculated as in 2.3.B.b (p. 61). The mean Φ^{Cl}/Φ^{Br} ratio was 1.0 during eight 30 minute periods in an open-circuited skin and 1.4 during four 30 minute periods in a short-circuited skin ($\varphi = 0$). In one experiment with a short-circuited skin, Φ_1^{Cl} and $-\Phi_2^{Br}$ were measured simultaneously. During the period before the addition of the drug, from the moment when the potential was kept at zero, there was a considerable difference in these flux values, which seemed to diminish after a time (cf. also p. 62). During four 30 minute periods when the action of a 0.1 mM solution of mersalyl was evident, the $-\Phi_2^{Cl}/0.97\,\Phi_1^{Br}$ ratio was 0.8.

Table 11.

Effect of mersalyl. Total conductance G, partial conductances, and ratios between them, for different groups of skins with Ringer's solution on either side. Mersalyl (0.1 mM) present in the inside solution. Mean values from each skin.

Experimental arrangement The values from each skin are the mean of 3—10 periods, each of 15 –30 minutes	G	G^{Cl}	$\frac{G_{aI}^{Na}}{G^{Cl}}$	$\frac{G_{aII}^{Na}}{G^{Cl}}$	$\frac{G}{G_{aII}^{Na}+G^{Cl}}$
1) *Open-circuited skins* ($\varphi \neq 0$).					
a) Values from 1 skin (10.3). Partial conductances calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	0.66	0.40	0.6	0.9	1.1
b) Mean values from 2 skins, including that in a). Partial conductance from Φ_1^{Cl}	0.66	0.39	1.0	—	—
2) *Short-circuited skins* ($\varphi = 0$).					
a) Values from 1 skin (10.2). Partial conductances calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	1.41	0.68	1.4	1.2	1.1
b) Values from 1 skin (9.2). Partical conductances calculated from Φ_{a2}^{Na} and Φ_2^{Cl}	0.96	0.50	1.0	1.0	1.0
c) Mean of the values of a) and b) (2 skins)	1.19	0.59	1.2	1.1	1.1
d) Mean values from 2 skins, including that in a). Partial conductance from Φ_1^{Cl}	1.38	0.56	1.7		
e) Mean values from 2 skins, including that in b). Partial conductance from Φ_2^{Cl}	1.05	0.55	1.0	—	
f) Mean of the values of d) and e) (4 skins)	1.21	0.56	1.4	—	
Mean of all experiments in open- and short-circuited skins	1.02	0.51	1.2	1.1	1.1

G values in k Ω^{-1} cm^{-2}. Cf. also Table 6.

c. ***Changes in the total and partial conductances of frog skin due to the action of mersalyl.*** The mean values of the total and partial conductances of different frog skins during the action of a 0.1 mM solution of mersalyl are given in Table 11. The first 15—30 minute period after the addition of the drug was omitted in the calculation of the mean values from each experiment. A better idea of the resistance changes during the action of mersalyl is given by the figures, however. Fig. 9 is quite typical. It is important to note that the sum of $G_{a\mathrm{II}}^{\mathrm{Na}}$ and G^{Cl} calculated as described in 2.3.B agrees well with the electrically measured G, the quotient $G/(G_{a\mathrm{II}}^{\mathrm{Na}} + G^{\mathrm{Cl}})$ being 1.1 (Table 11).

d. ***The influence of mersalyl on the active transport potential and the active transport of salt.*** At the same time as φ approaches zero, φ_a^{Na} and Φ^{NaCl} decrease to low values (see Fig 9). In experiments with short-circuited skin, I approaches zero (Fig. 10 and 11). In some cases φ changed its sign in open-circuited skins during the first period of action of the drug, and became a few mV negative. This may be explained by leakage of ions out of the skin, as it is not very likely that it is due to active transport of ions. When the formulas are used as described, however, a negative φ_a^{Na} is obtained. The mean Φ^{NaCl}, calculated according to eqn. (1.5.5.), was 199 $\mu\mu$mol cm^{-2} sec^{-1} before the action of mersalyl in two experiments, comprising respectively 5 and 6 periods of 15—30 minutes. During the action of the drug (0.1 mM), corresponding to 4 and 6 periods of 15—30 minutes in the same skins, it was 8 $\mu\mu$mol cm^{-2} sec^{-1}. The mean φ_a^{Na} changed during the corresponding periods from 74 mV to 5 mV.

B. The action of mersalyl on a skin with dilute Ringer's solution on the outside.

The results of a typical experiment in which a 0.1 mM solution of mersalyl acted on the inside of the skin are shown in Fig. 12. Measurements were made in the way described in

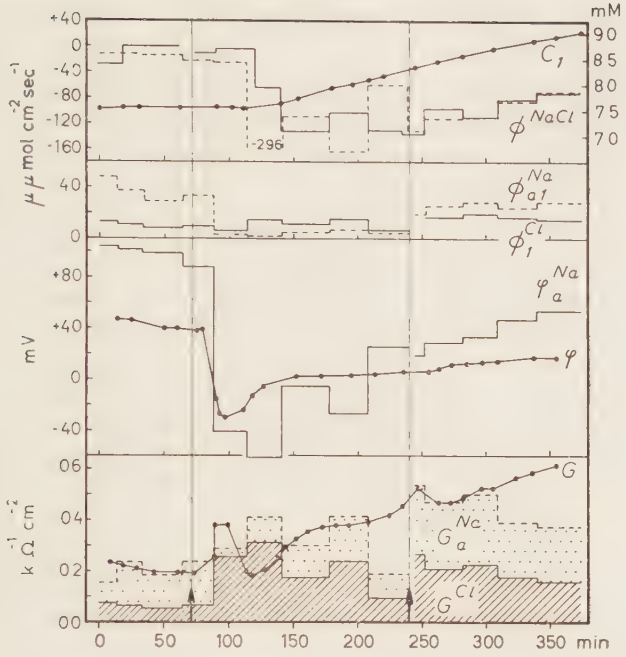

Fig. 12. Experiment 6.2. The effect of mersalyl on a skin with dilute solution on the outside. This experiment is similar to that described by Fig. 6. At the first arrow mersalyl was added to the inside solution to give a concentration of 0.11 mM. At the second, Ringer's solution was substituted for the inside solution, and the skin subsequently recovered.

2.3.C. The net flux of NaCl could thus be obtained in two ways, by eqns. (1.5.5) and (2.2.1). The experiment demonstrates that when Ringer's solution is substituted for the mersalyl containing Ringer's solution in 2 the skin recovers, at least partially, from the action of the drug. The effects of the drug on the different parameters of the skin which have been determined will be considered one after the other.

a. ***Changes in the conductance values.*** During the initial phase a rather typical tendency to a decrease in G may be discerned. Later there is a general increase in G, which continues after the removal of the drug from the inside solution. The mean $G/G_{aII}^{Na} + G^{Cl})$ ratio was 1.2 during 5 periods of 15 30 minutes of drug action.

Especially when the drug was beginning to act Φ_1^{Cl} and G^{Cl} were high, which may have been due to a leakage of ions out of the skin (or possibly to active transport of chloride ions?). Such behaviour seems to be rather a usual phenomenon, cf. Fig. 11.

b. ***Changes in the active transport potential of Na ions.*** The active transport potential, φ_a^{Na}, is seen to decrease rapidly when the drug action begins. The negative φ_a^{Na} may be looked upon as due to the (abnormally?) high G^{Cl} values (see above). The negative φ is compatible with the assumption that φ_a^{Na} approaches zero as a result of the action of the drug. The negative φ may then be looked upon as essentially a diffusion potential which requires $G_a^{Na} > G^{Cl}$, cf. eqn. (1.5.3). This condition may be fulfilled, cf. Table 11.

c. ***The net flux of salt.*** Before the action of the drug, Φ^{NaCl} is seen to be small. The active transport of Na ions balances approximately the electrochemical potential difference of the Na ions across the skin. During the action of mersalyl the Φ^{NaCl} becomes increasingly negative. As can be seen from Fig. 12, the two ways of calculating Φ^{NaCl} (from eqn. (1.5.5) and from (2.2.1), uncorrected for Φ^w) agree satisfactorily. During the period when the skin is recovering from the drug, it can be seen that at the same time as φ_a^{Na} increases Φ^{NaCl} becomes less negative.

In order to determine Φ^{NaCl} correctly from eqn. (2.2.1), Φ^w must be known. Possibly mersalyl might change Φ^w quite considerably from the value in »normal» skins (cf. 2.3.A). In one preliminary experiment Φ^w was measured as described in 2.3.A.d, before and after the addition of mersalyl in a concentration of 0.2 mM to the inside solution for a skin with an outside solution of about 5 mM NaCl. Before the addition of the drug, Φ^w was 4.1 μl cm^{-2} hr^{-1}. After mersalyl was added, Φ^w certainly decreased at the same time as the conductance increased, but measurements became difficult, as the permeability of the skin to water seemed to increase considerably. Water could be pressed slowly through the skin with only moderate

hydrostatic pressure. This was never possible with »normal» skins.

The decreased net flux of water may depend upon the fact that the skin becomes a less »semipermeable» membrane, but it may also be connected with an inhibition of a possible active transport of water. The low Φ^w after mersalyl justifies the use of values from (2.2.1) uncorrected for Φ^w.

C. Comments on the results with mersalyl.

This discussion will deal essentially with the correctness of the assumptions made in the treatment of the experimental data described above. Further discussion of the mode of action of the drug will be postponed to chapter 5.

As the permeability of the frog skin often changed continuously after the addition of the drugs, it is important to estimate how rapidly the diffusion processes may reach a new stationary state when a change has ocurred. In »normal» skins this process seems to be quite rapid. Even the first 15—30 minute period after the addition of the radioactive solutions to the compartment on one side of the skin often gave flux values which differed very little from those of the following periods, especially when the flux was measured inwards through the skin. Though the values from these first periods were not used in the results reported, the sum of G_{aII}^{Na} and G^{Cl} usually agreed quite well with G.

The results obtained after the addition of the diuretics referred to in this chapter seem to show that the time lag is small between the partial conductances estimated from the radioactive flux values and the electrically measured conductance of the skin (cf. the figures). A more important source of error seems to be a leakage of ions out from the skin, which probably accounts for some unexpectedly high flux values in connection with changes in the experimental condition of the skin.

Owing to the often rapid changes of the conductance of the skin, it seems doubtful whether the graphically obtained mean G values for a period are closely comparable with the partial

conductances calculated from the mean flux values during the same period. The results, however, seem to show that such comparison is possible with good approximation.

The experiments reported in 3.2.A, a and b, give some support to the assumption that the Cl ions are »passive» in the frog skin during the action of mersalyl. The decrease in the spontaneous resting potential of the skin or its ability to produce an electric current might depend on – besides a decrease in the active transport potential for Na ions – an active transport of Cl ions inwards through the skin. Such a transport might cancel the potential or current produced by the active transport of Na ions. The results already discussed are contrary to such a hypothesis.

Other indirect, though probably quite as reliable, evidence for the correctness of the assumption that Cl ions are passive in the skin is the good agreement between G and $G_{a\,\mathrm{II}}^{\mathrm{Na}} + G^{\mathrm{Cl}}$ (Table 11) when the latter are calculated on the assumption that Cl ions are passive. Reasoning analogous to that in 2.3.D.d shows that there cannot be a great deviation from the assumed behaviour of the ions with respect to active transport potentials.

If G_a^{Na} is calculated according to the hypothesis in 2.3.D.c, it is found that the mean G_a^{Na} from two open-circuited skins with $c_1 = c_2$ is 0.29 k Ω^{-1} cm^{-2} before and 0.02 k Ω^{-1} cm^{-2} after the action of mersalyl. In 4 short-circuited skins G_a^{Na} is 0.25 k Ω^{-1} cm^{-2} before and 0.28 k Ω^{-1} cm^{-2} during the action of mersalyl. The remarkable difference between the open- and short-circuited skins is difficult to explain, but the skins also behaved irregularly in other respects when q was kept at zero. In one experiment, 6.2, with $c_1 = 7$ –8 mM, G_a^{Na} was 0.10 k Ω^{-1} cm^{-2} before the drug, — 0.02 k Ω^{-1} cm^{-2} during the action of the drug, and 0.19 k Ω^{-1} cm^{-2} during the period when the skin was recovering from the drug action. Evidently mersalyl in many cases decreases the ability of the carrier to transfer Na ions.

To sum up, the essential effects of mersalyl seem to be a decrease in the ability of the postulated carrier system to transport Na ions, and at the same time a decrease in the active transport potential for Na ions. The general permeability of the skin to electrolytes is usually low at the beginning of the drug action, but later it tends to increase.

3.3. The effect of Aminophylline U.S.P. (Theophyllaminum Ph.S Ed XI).

Frog skin was found to be more sensitive to aminophylline when the drug acted on the inside than on the outside (LINDERHOLM, unpublished experiments). In the experiments reported here, 0.1 ml of a solution of aminophylline in Ringer's was added to the solution in 2 to give a concentration of about 50 mg/100 ml. Such a dose gave a certain effect though it was not much higher than the threshold dose. Tests were carried out to

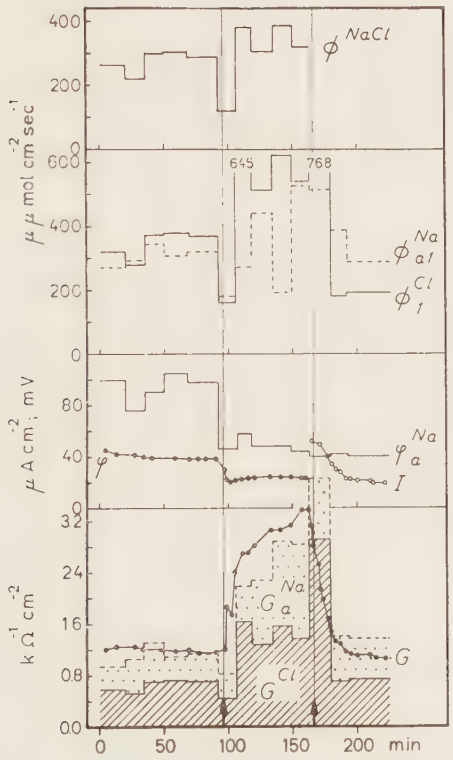

Fig. 13. Experiment 8.1. The effect of aminophylline on a frog skin with Ringer's solution on either side. At the first arrow aminophylline was added to the inside solution to give a concentration of 52 mg/100 ml. At the second arrow the skin was short-circuited ($\varphi = 0$). The subsequent decrease in G is remarkable. Inward fluxes of Na and Cl ions measured. The data are presented in the same way as in Fig. 9.

ensure that the change in pH brought about by alkaline aminophylline solution, was insignificant compared with the effect of the drug. The experiments were performed in a similar manner to those described earlier in connection with mersalyl, and the experimental data were also treated as before (see 2.3.B). An account of the different types of experiments performed is given below, under A and B.

A. Experiments with Ringer's solution of the same concentration on either side of the skin.

The results of some typical experiments are shown in Figs. 13, 14 and 15. As will be seen from the figures, the action of the drug begins almost immediately after it is added to the solution on the inside of the skin. In this respect the effect of amino-

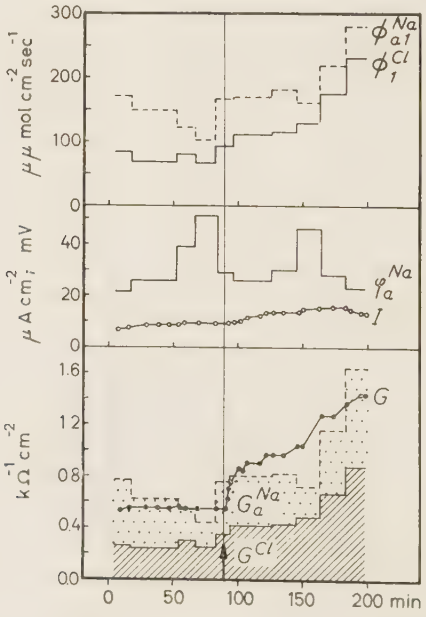

Fig. 14. Experiment 7.3. The effect of aminophylline on a short-circuited skin. The drug was added to the inside solution as indicated by the arrow to give a concentration of 52 mg/100 ml. Fluxes of Na and Cl ions measured inward through the skin. Ringer's solution on either side.

phylline differs from that of mersalyl. The main effect of aminophylline is an increase in the total and partial conductances and in ϕ^{NaCl}, while $q_{\prime\prime}^{Na}$ remains approximately unchanged.

a. ***Experiments made to test whether Cl ions behave as passive ions in frog skin during the action of aminophylline.*** Just as in the other experiments reported, it is of importance for the treatment of the data to know whether the Cl ions behave as passive ions during the action of the drug.

In some experiments in short-circuited skins the total flux of Cl ions was measured outwards and inwards in different skins. The results are summarized in Table 12. The skins are unfortunately rather dissimilar, and the flux ratio may be regarded as very uncertain. However, when compared with the flux values for Na ions in the same experiments the results seem to give some support to the assumption that Cl^- behaves as a passive ion.

Table 12.

Effect of aminophylline. Fluxes of Cl and Na ions inward and outward through different short-circuited skins ($\varphi = 0$), the total conductance G, and the current through the skin I, with aminophylline (50 mg/100 ml) present in the inside solution. Mean values from each experiment.

Exper. no.	n	Φ_1^{Cl}	G $k\Omega^{-1}$ cm^{-2}	Exper. no.	n	$-\Phi_2^{Cl}$	G $k\Omega^{-1}$ cm^{-2}	$\frac{-\Phi_2^{Cl}}{\Phi_1^{Cl}}$
8.1	3	380	1.6	9.4	5	264	1.9	0.7
14.2	4	155	1.2	12.2	3	317	2.3	2.0
Mean		268	1.4	Mean		290	2.1	1.4

Exper. no.	n	Φ_{a1}^{Na}	I/F	Exper. no.	n	$-\Phi_{a2}^{Na}$	I/F	$\frac{-\Phi_{a2}^{Na}}{\Phi_{a1}^{Na}}$
8.1	3	399	302	9.4	5	77	396	0.2

Flux values in $\mu\mu$mol cm^{-2} sec^{-1}, I/F in $\mu\mu F$ cm^{-2} sec^{-1}.
n is the number of 15—30 minute periods included in each experiment.

b. ***Experiments with Cl^{36} and Br^{82} in combination to test whether these ions behave as passive ions during the action of aminophylline.*** In two experiments, the fluxes of Cl^{36} and Br^{82} ions were measured in the same direction simultaneously.

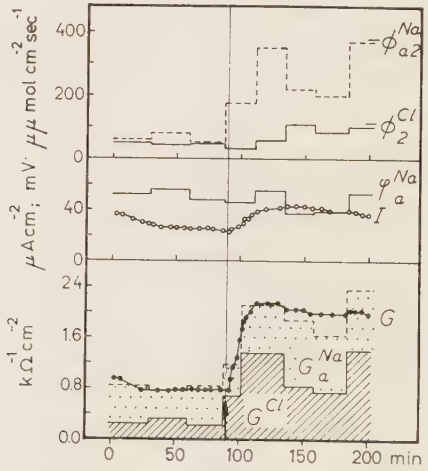

Fig. 15. Experiment 9.4. An experiment similar to that given in Fig. 14. Fluxes of Na and Cl ions measured outward through the skin. Aminophylline concentration 50 mg/100 ml.

The fluxes were calculated in the same way as in 2.3.B. b and 3.2.A.b. The resulting mean Φ^{Cl}/Φ^{Br} ratio was 1.3 in an open-circuited skin and 0.6 in a short-circuited skin during the action of aminophylline (50 mg/100 ml) on the inside.

In one experiment with a short-circuited skin $-\Phi_2^{Cl}$ and Φ_1^{Br} were measured simultaneously. During three 30 minute periods of drug action the $-\Phi_2^{Cl}/0.97\ \Phi_1^{Br}$ ratio was 0.9. This value is of the same magnitude as that obtained in a similar experiment with mersalyl.

c. ***Total and partial conductance during the action of aminophylline.*** Mean values of total and partial conductances of different frog skins during the action of aminophylline in a concentration of 50 mg/100 ml are summarized in Table 13.
If these values are compared with those of »normal« skins (Table 6) and skins influenced by mersalyl (Table 11), it is

Table 13.

Effect of aminophylline. Total conductance G, partial conductances and ratios between them, for different groups of skins with Ringer's solution on either side. Aminophylline (50 mg/100 ml) present in the inside solution. Mean values from each skin.

Experimental arrangement The values from each skin are the mean of 3—6 periods, each of 15—30 minutes	G	G^{Cl}	$\frac{G_{aI}^{Na}}{G^{Cl}}$	$\frac{G_{aII}^{Na}}{G^{Cl}}$	$\frac{G}{G_{aII}^{Na}+G^{Cl}}$
1) *Open-circuited skins* ($\varphi \neq 0$)					
a) Values from 1 skin (8.1). Partial conductances calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	2.75	1.25	1.7	0.9	1.5
b) Mean values from 2 skins, including that in a). Partial conductance from Φ_1^{Cl}	1 93	0.80	2.1	—	—
2) *Short-circuited skins.* ($\varphi = 0$).					
a) Mean values from 2 skins (7.3, 8.1). Partial conductance calculated from Φ_{a1}^{Na} and Φ_1^{Cl}	1.32	1.00	0.8	0.9	1.0
b) Values from 1 skin (9.4). Partial conductances calculated from Φ_{a2}^{Na} and Φ_2^{Cl}	1.92	1.01	1.0	0.9	1.1
c) Mean of the values of a) and b) (3 skins)	1.62	1.01	0.9	1.0	1.0
d) Mean values from 3 skins, including those in a). Partial conductance from Φ_1^{Cl}	1.12	0.81	1.5	—	—
e) Mean values from 2 skins, including that in b). Partial conductance from Φ_2^{Cl}	2.09	1.11	1.2	—	—
f) Mean of the values of d) and e) (5 skins)	1.61	0.96	1.4	—	—
Mean of all experiments in open- and short-circuited skins	1.76	0.95	1.4	0.9	1.1

G values in $k\Omega^{-1}$ cm^{-2}. Cf. also Table 6.

evident that aminophylline greatly increases the total and partial conductances of the frog skin. The sum of $G_{a\,\mathrm{II}}^{\mathrm{Na}}$ and G^{Cl} calculated on the assumption that Cl^- is a passive ion agrees well with the electrically measured G for skins under the influence of aminophylline, the mean $G/(G_{a\,\mathrm{II}}^{\mathrm{Na}} + G^{\mathrm{Cl}})$ ratio being 1.1 (cf. Table 13).

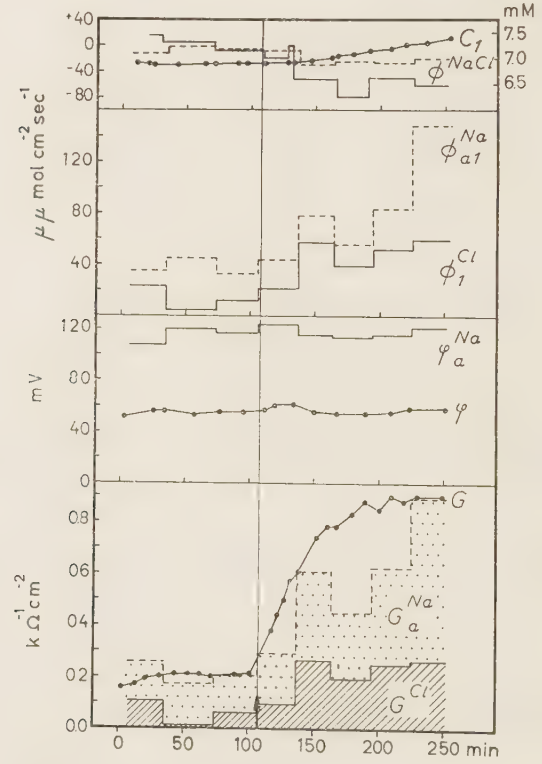

Fig. 16. Experiment 5.2. The effect of aminophylline on a skin with a dilute solution on the outside. Experimental arrangement similar to that of experiments 5.1 (Fig. 6) and 6.2 (Fig. 12). Aminophylline was added as indicated by the arrow, to give a concentration of 53 mg/100 ml in the inside solution. Fluxes of Na and Cl ions measured inward through the skin.

d. ***The effect of aminophylline on the active transport potential and the active transport of salt.*** The active sodium transport potential $\eta_{\,\mathrm{II}}^{\mathrm{Na}}$ is, as already mentioned, essentially unaffected

by aminophylline. In 2 open-circuited skins the mean φ_a^{Na} was 65 mV and in 5 short-circuited skins it was 30 mV during the action of the drug. These values are slightly lower than in »normal» skins. In one experiment there was even a slight increase in φ_a^{Na}.

The almost unchanged φ_a^{Na}, combined with the increase in G_a^{Na} and G^{Cl}, will increase the transport of NaCl in these experiments (cf. 1.6.b.). When calculated according to eqn. (1.5.5.) the mean Φ^{NaCl} was 288 $\mu\mu$mol cm^{-2} sec^{-1} during the action of aminophylline in two experiments comprising respectively 5 and 6 periods of 15 to 30 minutes. Before adding the drug, it was 174 $\mu\mu$mol cm^{-2} sec^{-1} for 4 to 6 periods of 15 to 30 minutes in each skin. The mean φ_a^{Na} was 87 mV before and 65 mV after the action of the drug.

B. An experiment demonstrating the action of aminophylline on a skin with dilute Ringer's solution on the outside.

The results of a typical experiment are given in Fig. 16. It was performed in a similar manner to other experiments with a low c_1 (see 2.3.C and 3.2.B). Aminophylline (50 mg/100 ml) acted on the inside of the skin.

a. *Changes in the conductance values.* In this experiment the total and partial conductance values increased to about three times the values for »normal» skins. The increase is still more marked than in the experiments with $c_1 - c_2$. The mean $G/(G_{aII}^{Na} + G^{Cl})$ ratio is 1.4 from 5 periods of 15 – 30 minutes.

b. *Changes in the active transport potential and the net transport of salt.* The active transport potential is seen to have the same value before and after the action of the drug. In spite of this fact, Φ^{NaCl}, which was slightly negative before the action of the drug commenced, becomes increasingly negative. This is in agreement with the reasoning in 1.6.b.

After the addition of aminophylline, the agreement between Φ^{NaCl} as calculated from eqn. (1.5.5.) and that obtained from

eqn. (2.2.1) is not so good (see Fig. 16). The latter flux values in the figure are not corrected for Φ^w.

In an experiment of the same kind as that described in 3.2.B.c, Φ^w was measured in a skin with a dilute NaCl solution on the outside, before and after the action of aminophylline. As in the case of mersalyl, Φ^w decreased after the addition of aminophylline (100 mg/100 ml) to the inside solution. At the same time as the conductance of the skin increased, the skin seemed to be more permeable to water than before, because only a moderate hydrostatic pressure was required to force water slowly through the skin. This experiment indicates that Φ^w is too small after aminophylline to account for the difference between the Φ^{NaCl} values. However, the difference may be explained by leakage of electrolytes out from the skin.

C. Comments on the results with aminophylline.

Like that in 3.2.C, this discussion is essentially intended to show that the assumptions made for the treatment of the experimental data are approximately correct. The results will be further discussed in Chapter 5.

It is conceivable that the marked increase in current production by the skin after aminophylline might be due to an active transport of Cl ions from 2 to 1, i.e. in the opposite direction to the transport of Na ions. Such an effect would also explain the maintenance of the potential across the skin in spite of the considerably increased conductance. The assumption is not very likely, however, in view of the results of the Cl flux measurements given in Table 12, or those obtained with the combined use of Cl^{36} and Br^{82} (3.3.A.b).

On the contrary, these results support the assumption that Cl^- behaves as a passive ion in frog skin during the action of aminophylline (cf also 3.3.A.a). They show an approximate agreement with the requirements which eqn. (1.2.1) imposes on a passive ion.

The good agreement between G and $G_a^{Na} + G^{Cl}$ mentioned in 3.3.A.c. and 3.3.B.a is probably still better evidence for the

passive behaviour of Cl^-, and renders a marked deviation from this behaviour unlikely (cf. 2.3.D.d and 3.2.C.).

If G_a^{Na} is calculated according to the hypothesis put forward in 2.3.D.c., we obtain a mean G_a^{Na} from two open-circuited skins of 0.16 k Ω^{-1} cm^{-2} before and 0.61 kΩ^{-1} cm^{-2} during the action of aminophylline. In 4 short-circuited skins the corresponding values were 0.241 and 0.292 k Ω^{-1} cm^{-2}. In this case, as after mersalyl, there is a noticeable difference in the G_a^{Na} values in open- and short-circuited skins. In experiment 5.2, Fig. 16, with $c_1 \approx 7$ mM, G_a^{Na} was 0.09 k Ω^{-1} cm^{-2} before and 0.39 k Ω^{-1} cm^{-2} during the action of aminophylline. It thus seems as if aminophylline increases the ability of the carrier to transport Na ions.

During the action of the drug φ_a^{Na} is about 110 mV both for short-circuited and open-circuited skins with $c_1 = c_2$, and 160 mV in experiment 5.2, with $c_1 \approx 7$ mM.

To sum up, the effect of aminophylline seems to be a general increase in the permeability of the skin to electrolytes, and an increase in the capacity of the postulated sodium carrier system to transfer Na ions, at the same time as the active transport potential remains essentially unchanged.

3.4. The effect of a change in pH induced by CO_2.

By bubbling a mixture of 5 per cent CO_2, 95 per cent O_2 through the Ringer's solutions instead of air, the pH of the solutions decreased. Earlier experiments had shown that this was accompanied by considerable changes in the electrical potential and conductance of the frog skin, which were reversible when air was again bubbled through the solutions. Only 3 such experiments have been performed with the combined use of radioactive isotopes and electrical methods. A brief account of these experiments is given below.

A. Experiments with solutions of the same concentration on each side of the skin.

The results of such an experiment are shown in Fig. 17. In another similar experiment only the Cl flux was measured.

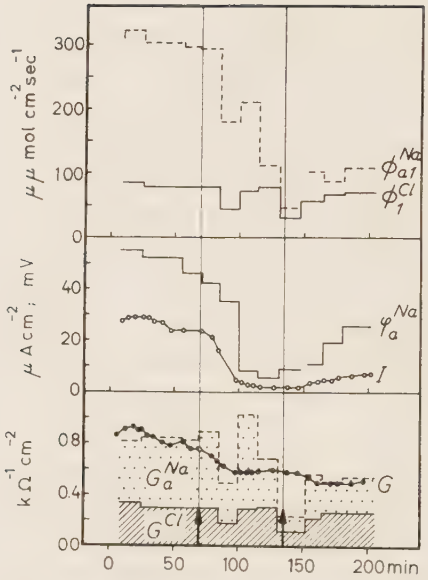

Fig. 17. Experiment 7.2. The effect of a pH change induced by bubbling a 5 per cent CO_2, 95 per cent O_2 gas mixture through the bathing solutions. At the first arrow there was a change from air bubbling to the CO_2-O_2 mixture, and at the second arrow air bubbling recommenced. Ringer's solutions on either side. Fluxes of Na and Cl ions measured inward through the skin.

When the CO_2-O_2 mixture was bubbled through the phosphate Ringer, its pH decreased to about 6.5. As may be seen from Fig. 17, it takes a considerable time — about a half to one hour — before the full effect of bubbling CO_2 is attained. It takes about the same time for bubbling air to restore the skin. This delayed response seems to run approximately parallel to the pH changes of the bathing solutions. The values considered to be characteristic of CO_2 periods are therefore taken from the interval from about 30 minutes after the beginning of the CO_2 bubbling until about 30 minutes after the resumption of air.

The effect of the CO_2 may be briefly summarized as a decrease in φ or the electrical current production by the skin, and, at the same time, usually a decrease in G. φ_a^{Na} also decreases. Though it is not so obvious from the results shown in Fig. 17, the other experiment showed a marked decrease in

the G_a^{Na} relative to G^{Cl}. The mean $G/(G_{aII}^{Na} + G^{Cl})$ ratio in one skin while CO_2 was bubbled through the solution (4 periods of 15—30 minutes) was 1.1, which seems to indicate that the assumptions made, and the equations used, are valid in this case also.

B. The effect of CO_2 on a skin with a dilute solution on the outside.

The results of such an experiment are shown in Fig. 18. The experimental arrangement was the same as in the other experiments with a low c_1 (see 2.3.C, 3.2.B. and 3.3.B.). By bubbling the 5 per cent CO_2, 95 per cent O_2 mixture through the bicarbonate Ringer's solution, which had a pH 8.1 –8.2 with air bubbling, its pH changed to about 6.5. At the same time, φ, φ_a^{Na} and G decreased and so did the inward fluxes. The mean $G/(G_{aII}^{Na} + G^{Cl})$

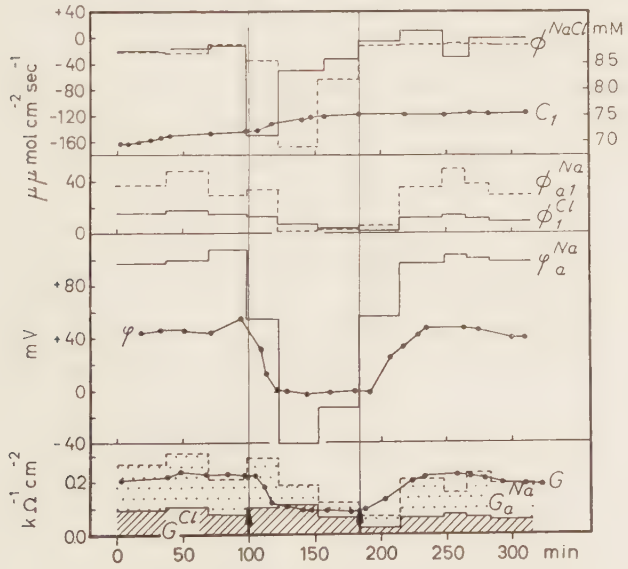

Fig. 18. Experiment 6.1. The effect of a pH change induced by bubbling a 5 per cent CO_2, 95 per cent O_2 mixture through the solutions bathing a frog skin, with dilute solution on the outside. At the first arrow bubbling with the CO_2-O_2 mixture was began, and at the second arrow it was interrupted and air again bubbled. Experimental arrangement as in the experiments described by Fig. 6, 12, and 16.

ratio was 1.0 during 3 periods of 15—30 minutes. Φ^{NaCl} became more negative during the period of CO_2 bubbling, though the decreased conductance tended to decrease all fluxes. Approximately one hour after resuming air bubbling the skin recovered.

C. Comments on the results with CO_2.

It seems possible to apply the same treatment as in earlier sections to the results of these experiments with CO_2. The fairly good agreement between G and the sum of $G_{a\,II}^{Na}$ and G^{Cl} is again the best evidence that the ions behave as assumed at a low pH and a high CO_2 partial pressure.

The results concerning the active transport of Na ions seem to be in agreement with observations on the effect of a high CO_2 partial pressure and low pH made by Ussing (1949 b) and Ussing and Zerahn (1951).

The general decrease in G seems to be a characteristic consequence of a decrease in pH, but it is possible to obtain such a decrease in a great many other ways, for instance in connection with a lack of oxygen (Marsh 1947). It may be that it is related to some change in the hypothetical sodium carrier molecule.

If G_a^{Na} is calculated according to the hypothesis in 2.3.D.c. (p. 78) it is found (in experiment 7.2, Fig. 17) to be 0.30 $k\Omega^{-1}$ cm^{-2} before the commencement of CO_2 bubbling and 0.16 $k\Omega^{-1}$ cm^{-2} when the effect of CO_2 was quite definite. In another experiment even negative G_a^{Na} values were obtained. In experiment 6.1, Fig. 18, G_a^{Na} was 0.24 $k\Omega^{-1}$ cm^{-2} before CO_2 bubbling and — 0.02 $k\Omega^{-1}$ cm^{-2} during CO_2 bubbling.

It thus seems as if CO_2 or the decrease in the pH of the solutions decreases the ability of the carrier system to transfer Na ions, at the same time as the active transport potential decreases to low values. In this respect, the action of CO_2 seems to be similar to that of mersalyl.

Chapter 4.

The effect of Mersalyl, $HgCl_2$, Aminophylline, and variation in pH on the oxygen uptake of frog skin.

4 1. Earlier work on the relation between oxygen consumption and the electrical potential or current production by frog skin and some factors which influence them.

The relationship between the oxygen uptake and the electrical potential or current maintained by frog skin has been extensively investigated. Two important papers which review this work, by DEAN and GATTY (1937) and LUND (1947), have been referred to earlier (cf. 2.1.a).

As a rule, it has been found that factors which decrease the oxygen uptake also decrease the electrical potential across the skin. FRANCIS (1934) found that the curves relating potential and rate of respiration to oxygen concentration were almost identical. In oxygen-free media the potential falls to low values in 20– 40 minutes (FRANCIS and PUMPHREY 1933). Inhibitors of the cytochrome system such as NaCN, Na_2S and CO, and dehydrogenase inhibitors such as aliphatic alcohols and urethan, decrease the potential (LUND 1928, BOELL and TAYLOR 1933, FRANCIS 1934, TAYLOR 1935, FRANCIS and GATTY 1938).

HUF (1935, 1936 a, b) demonstrated the close relation between metabolism and the active transport of substances across the skin. As previously mentioned (2.1.a), he found that the potential and the active transport were inhibited by monobromoacetate but could be restored by lactate and pyruvate. Later, FRANCIS and GATTY (1938) showed that the potential of iodoacetate poisoned skin could be restored by acetate, propionate and butyrate, besides lactate and pyruvate. Thus, when the potential is regarded as a result of the active transport of sodium ions, it seems that a hydrogen donator is necessary for the work of the active transport mechanism, together with dehydrogenase and cytochrome enzymes. All these facts taken together seem

to be in conformity with the idea that a redox pump like that proposed by CONWAY (1951) functions in frog skin (cf. 2.3.D.a.).

Deviation from the parallelism between the potential and the respiratory rate have been reported. The potential is affected more than the oxygen consumption by some substances, such as carbamates, iodoacetate, CO, urethane, ouabaine, while saponin and bile salts nullify the potential without reducing the oxygen uptake at all (PONDER and MACLEOD 1937, TAYLOR 1935, FRANCIS and GATTY 1938). Permanganate increases the oxygen uptake and depresses the potential (DEAN and GATTY, quoted from FRANCIS and GATTY 1938). Several of the substances mentioned are known to have a marked influence on the permeability of frog skin. It is possible to explain their effect in terms of the intimate connection between the potential and the permeability to electrolytes which has been discussed in earlier chapters.

ADOLPH (1929) showed that 0.005 M $HgCl_2$ completely inhibited the O_2 uptake of frog skin. $HgCl_2$ is also known to depress the potential (cf. 3.1), but so far, there appear to be no figures which make it possible to estimate whether the two processes are inhibited at the same concentration or whether they have different sensitivities to $HgCl_2$. The effect of organic mercury compounds (diuretics) or xanthine derivatives on the oxygen uptake of frog skin does not seem to have been investigated previously.

It may be possible to learn something about the nature of the »sodium pump« by studying factors which affect either or both potential and respiration. Measurements of just the electrical potential across the skin provides little information about the »sodium pump«, and such measurements should be accompanied by a determination of the active sodium transport potential and the total and partial conductances of the skin. It may then be possible to distinguish factors affecting the »sodium pump« more specifically from factors producing their effects by a general deterioration of cell membranes or inhibition of metabolic processes.

The results reported in the preceding chapters tell us something about the effects of mersalyl, aminophylline and a

low pH on the ability of the 'sodium pump' to perform work. The experiments reported in this chapter were performed in order to find out more about their general effects on cell activities.

4.2. Methods.

a. ***Manometric method.*** It was desirable to have the conditions for the skin in these experiments as far as possible similar to those in the experiments where the active sodium transport and the permeability properties of the skin were investigated. The Warburg constant volume type of respirometer was used to measure the oxygen uptake by the direct method. CO_2 was absorbed by alkali (10 per cent, in some experiments 20 per cent NaOH). The low partial pressure of CO_2 (p_{CO_2}) which is necessary in the direct Warburg method was no disadvantage, as it differs little from the p_{CO_2} in the experiments with radioactive isotopes in Chap. 2 and 3, where air was bubbled through the bathing solutions.

For technical details, DIXON (1943) and UMBREIT, BURRIS and STAUFFER (1945) were consulted. The temperature of the water bath was 21°.3 C ± ± 0°.1 C (range). Readings of the manometers were taken every 15—20 minutes. The horizontal shaking had a frequency of about 100 oscillations per minute with an amplitude of 5 cm. The volumes of the manometer flasks were determined by weighing them with mercury.

b. ***Animals.*** The experiments were performed in September and October. The frogs (*Rana temporaria*) were kept as described earlier in 2.2.a. They are usually smaller than those used in the experiments with radioactive isotopes.

c. ***Immersion fluids.*** The immersion fluid was made up from (i) 1 liter of Ringer's solution containing 111.5 mM NaCl, 2.68 mM KCl, 3.60 mM $CaCl_2$ and 1 g glucose per liter, and (ii) 2.2 ml of a 0.5 M phosphate buffer, pH 7.5. This solution contains less phosphate buffer than that used in the experiments with radioactive isotopes with the same concentration on either side of the skin, but it had been used earlier in many experiments in which the effect of diuretics on the potential and conductance of the skin was studied. In 8 experiments the immersion fluid was made up from (i) 3 parts of the above mentioned Ringer's solution and (ii) 1 part 0.1 M phosphate buffer (pH 7.5). This better buffered solution was used in some experiments with high concentrations of mersalyl and aminophylline, the solutions of which are alkaline. To judge by these experiments, the variation of the amount of phosphate buffer within the range used did not influence the results. All substances used for preparing the solutions were of reagent grade and the distilled water was obtained from a tinned boiler and kept in a stainless steel vessel.

Table 14.

The effect of mersalyl, $HgCl_2$, aminophylline and changes in pH on the O_2 uptake of frog skin. The O_2 uptake was measured in phosphate Ringer (pH 7.5) over a control period of about 100 minutes. Then the different drugs were added, or the change in pH induced by addition of HCl or NaOH, and the O_2 consumption determined for the following two 100 minute periods.

Symbols in the table.

Q_{O_2} (0—100) the average O_2 uptake during the first 100 minutes before the addition of drugs or changes in pH. (μl O_2 NTP hr^{-1} mg^{-1} of dried tissue). The dried weight was 16 per cent of the wet weight with a coefficient of variation = 17 per cent.

ΔQ_{O_2} (100—200) percentage difference between the mean Q_{O_2} during the first 100 minutes of the action of the drug and Q_{O_2} (0—100).

ΔQ_{O_2} (200—300) percentage difference between the mean Q_{O_2} during the period 200—300 minutes after the beginning of the experiment and Q_{O_2} (0—100).

M mean of each experimental group.

n number of experiments in each group.

s^2 variance.

t for the meaning of »t», see Fisher (1944).

P the probability that the differences are caused by random factors. The P values were obtained from Fisher and Yates (1943).

Table IV.

Drug concentration or pH in the resperatory vessel after the addition of 0.1 ml of different drug-solutions or HCl or NaOH to the phosphate-Ringer	n	Q_{O_2} (0—100)		ΔQ_{O_2} (100—200) in per cent of Q_{O_2} (0—100)				ΔQ_{O_2} (200—300) in per cent of Q_{O_2} (0—100)			
		M	s^2	M	s^2	t	P	M	s^2	t	P
Mersalyl-conc. in mM											
2.77	4	—0.61	0.035	+98	1827	4.58	0.02	+49	659	3.81	0.05
1.03	3	—0.88	0.010	+44	444	6.06	0.05	+4	161	0.39	0.80
0.21	6	—1.15	0.094	+56	968	4.41	0.01	+31	2214	1.63	0.20
0.10	5	—0.64	0.026	+63	533	6.11	0.01	+45	1130	2.98	0.05
0.010	3	—0.77	0.018	— 6	79	1.19	0.40	—18	28	5.74	0 05
$HgCl_2$-conc. in mM											
1.00	3	—0.86	0.223	—68	71	14.10	0.01	—80	87	15.00	0.01
0.20	3	0.91	0.073	15	362	1.37	0.40	—37	274	3.94	0.10
0.10	7	—0.67	0.039	+ 7	436	0.87	0.50	+16	777	1.56	0.20
0.050	4	—0.89	0,019	— 8	645	0 65	0.60	— 3	487	0.30	0.80
0.010	2	—0.77	0.017	—12	269	1.08	0.50	— 6	253	0.57	0.70
Theophyllamin, conc. in mg/100 ml											
322 (14.78)[1]	3	—0.68	0.226	+77	2033	2.96	0.10	+17	665	1.11	0.40
97.0 (4.27)	6	—1.05	0.072	+65	1241	4.53	0.01	+67	1557	4.17	0.01
32.0 (1.44)	4	—0.69	0.042	+50	306	5.76	0.02	+55	1018	10.80	0.01
9.70 (0.43)	5	—1.25	0.052	+11	846	0.87	0.50	+15	809	1.18	0.20
Variation in pH											
pH = 6.0—6.5	9	—0.73	0.088	—24	61	9.35	0.001	—39	62	15.10	0.001
6.5—7.0	3	—0.58	0.013	—36	17	15.20	0.01	—22	72	4.52	0.05
8.6—8.0	7	—0.88	0.058	— 1	275	0.19	0.90	+ 6	647	0.60	0.60
9.0—8.0	6	—0.83	0.031	— 6	507	0.63	0.60	+ 3	304	0.42	0.70
9.5—8.0	3	—0.93	0.079	+21	236	2.40	0.20	+ 6	4	5.36	0.05
10 5—9.6	3	—0.79	0.015	+24	346	2.24	0.20	+38	532	2 86	0.20
Controls, pH = 7.5	31	—0.85	0.081	— 6	164	2.34	0.05	— 8	258	2.79	0.01

[1] The figures within brackets give the concentration of theophylline in m M when aminophylline is assumed to contain 80 per cent of anhydrous theophylline.

d. *Experimental procedure.* About 100 mg (wet weight) of abdominal skin, and sometimes also skin from the thighs, was cut into two or three pieces and used in each respirometer. The skin was quickly dried with filter paper before adding it to the 3 ml of immersion fluid in the manometer vessel. Measurements began about one hour after the frog was killed and the skin removed. By this time the O_2 consumption should have reached a steady value (FRANCIS 1934). The substances to be investigated were added to the side bulb of the vessel, dissolved in a volume of 0.1 ml. In the side bulb of the control vessel 0.1 ml Ringer was added. The manometers were filled with oxygen. According to FRANCIS (1934), the O_2 consumption is the same in air as in pure oxygen. At partial pressures of O_2 lower than that of O_2 in air the O_2 consumption begins to fall off, but only att the lowest O_2 pressures is the diffusion into the tissue the limiting factor for the respiration of the skins, which are about 0.2—0.3 mm thick. After equilibration for about 20 minutes the Q_{O_2} was determined during the first 100 minutes. After this control period the content of the side bulb was run into the immersion fluid, and the experiment was continued for another 200 minutes.

At the end of the experiment, the pH of the bathing fluid was determined by using a glass electrode. The pieces of skin were quickly dried on filter paper, placed in weighing vessels and the wet weight determined. Then the skin was dried at 110°C for 15 hours and the dry weight determined to the nearest 0.1 mg.

4.3. Results obtained from measurements of the O_2 uptake.

a. *Controls.* (»Normal skins».) The results are given in Table 14 and in Figs. 19 and 20. The mean Q_{O_2} from 116 experiments on skin from 31 frogs was -0.85 ± 0.077 μl O_2 NTP hr^{-1} mg^{-1} dry weight (mean ± standard error of the mean) during the first 100 minutes of measurement. During the following 100 minutes periods the Q_{O_2} decreased slightly in the 31 control experiments (one for each experimental day). The decrease is of the same order of magnitude as that found by FRANCIS (1934) and TAYLOR (1935) — about 3 per cent per hour.

b. *Experiments with mersalyl.* In the concentration range 0.1—2.7 mM, mersalyl increases the oxygen uptake by about 50 per cent (Table 14) during the first 100 minutes. During the subsequent 100 minutes the increase, as compared with the control period, seems smaller.

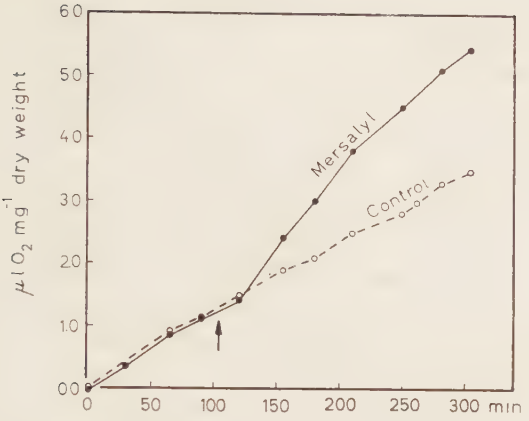

Fig. 19. A typical experiment which shows the effect of a 0.1 mM solution of mersalyl on the O_2 uptake of frog skin.

c. ***Experiments with*** $HgCl_2$. In concentrations above 0.2—1.0 mM, $HgCl_2$ inhibits the oxygen uptake (Table 14). It may be that $HgCl_2$ increases the oxygen uptake at a concentration of 0.1 mM, but too few experiments were performed to give this result a high degree of probability. Below 0.05 mM, $HgCl_2$ seemed to have an insignificant effect on the respiration.

d. ***Experiments with aminophylline.*** Aminophylline seems to increase the oxygen consumption of frog skin in the concentration range 9.7—322 mg/100 ml, though the results are statistically significant only in the range 32—97 mg/100 ml (Table 14). The increase is about the same in the two successive 100 minute periods after the addition of the drug.

e. ***The effect of changes in pH on the oxygen uptake.*** A decrease in pH induced by adding a small amount of HCl decreases the oxygen uptake significantly (Table 14). The pH values in the table give the range of pH variation in the different experimental groups. The pH value usually changed slowly but continuously during the experiments, as was found by interrupting some experiments before the termination of the usual 300 minutes. This change was significant, especially in the experi-

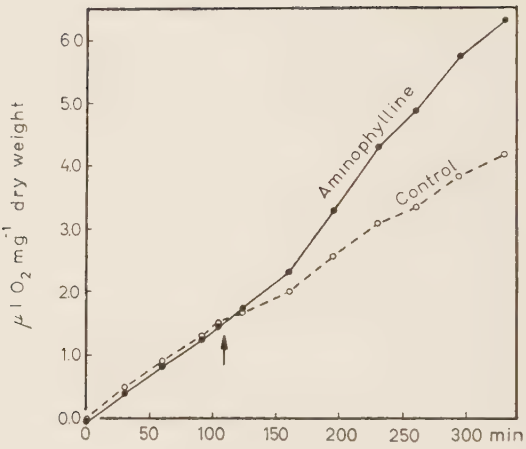

Fig. 20. A typical experiment which shows the effect of aminophylline (42 mg/100 ml) on the O_2 uptake of frog skin.

ments in which NaOH was added to increase the pH, and often amounted to about 1 pH unit. The change was always towards the neutral point.

When sufficient NaOH is added to increase the pH to about 10 at the beginning of the experiment, there seems to be an increase in the oxygen uptake. A smaller change seems to have little influence on the oxygen uptake.

4.4. Comments on the results.

The Q_{O_2} values obtained for the control periods are in the range of those reported by others (cf. for instance ADOLPH 1929, PONDER and MACLEOD 1936).

An interesting result is that mersalyl and $HgCl_2$ have so different effects on the respiration. They seem to be active in about the same molar concentrations. About 0.1 mM of mersalyl begins to stimulate the respiration, and in the same molar concentration range $HgCl_2$ may have a similar action. It may be that this possible stimulating effect of $HgCl_2$ is hidden at higher concentrations by an inhibitory action of $HgCl_2$ on some other metabolic process which is not affected by higher con-

centration of mersalyl. This will be discussed further in the next chapter. The results do not accord with those reported by MUDGE (1951) for the action of mersalyl (salyrgan) and $HgCl_2$ on rabbit kidney slices. In that tissue both mersalyl and $HgCl_2$ seem to inhibit respiration in concentrations of about 1 mM.

Aminophylline also increases the respiratory rate. Since both the mersalyl and aminophylline solutions are alkaline and their addition at some of the higher concentrations increases the pH in some experiments — though by less than 1 pH - unit — it is conceivable that the change in pH could itself bring about the change in the respiration. The experiments in which varying amounts of Na OH were added to increase the pH are definitely contrary to this supposition, as a much greater change in pH seems to be required to change Q_{O_2} significantly. Whether it is the increased pH as such or the increase in carbonate ions which may be expected in the alkaline medium which affects the respiration is beyond the scope of this discussion. A low pH, on the other hand, inhibits the respiratory rate.

Chapter 5.

General discussion of the results obtained.

5.1. The relation between active sodium transport and the oxygen consumption of frog skin, and the effects of the diuretic drugs studied.

A. Experiments with "normal" skins.

a. ***Total energy released by oxidation processes.*** The mean oxygen uptake of frog skin was 0.85 μl hr^{-1} mg^{-1} dry weight. In order to compare this figure with the results from the experiments with radioactive isotopes, it is necessary to determine the dry weight per cm^2 of skin.

This was done for ten skins which had been mounted in the frog skin chamber. The surface of the skin which covered the hole between the two half cells was cut out. Its area should have been 7.1 cm^2; this was checked by laying it out on paper, cutting out the paper corresponding to the skin area, and measuring the area cut out. The mean of 13 such measurements was 7.2 cm^2. The wet and dry weight of the skins were determined as described (4.2.d). The dry weight of these skins was 19 per cent of the wet weight.

It was found that 1 cm^2 of skin corresponded to 5.6 mg dry weight. Thus the mean O_2 consumption of the skin is 4.8 μl cm^{-2} hr^{-1}. This value agrees fairly well with a mean value of 5.2 μl cm^{-2} hr^{-1} that may be calculated from the results with bullfrogs reported by LUND and STAPP (1947). If the RQ of frog skin is between 0.72 (DOLK and POSTMA 1927) and 0.97 (BORNSTEIN and KLEE 1927) and the corresponding caloric equivalents of oxygen are 4.70, to 5.01 cal per ml O_2, it will be found that the energy released by oxidation processes is 6.3 to 6.7 μcal cm^{-2} sec^{-1}, which is about 30 per cent lower than the value derivable from the results given by FRANCIS (1934) for summer frogs. Expressed in electrical units, the energy released is about 27 μwatt cm^{-2}.

b. ***The minimum energy required for the active transport process.*** Attempts have been made earlier to calculate the efficiency of various biological objects as sources of electrical energy. Some of the relevant literature has been referred to previously, 2.1. The review by CRANE (1950) contains many references, especially to work on gastric mucosa. These calculations are usually only rough approximations of the energy used for active transport of ions, owing to the incomplete knowledge of essential electrical characteristics of the object.

The minimum energy output of frog skin for the sodium transport can be calculated from the results given in 2.3. We will do this for skins with Ringer's solution of the same concentration on either side during periods when they were (i) short-circuited ($\varphi = 0$) and (ii) open-circuited.

Short-circuited skins. The mean electrical current in 10 skins (in 5 skins the radioactive fluxes were measured inward and in 5 outward through the skin) was 197 $\mu\mu F$ cm^{-2} sec^{-1} or 19 μA cm^{-2}. The mean G_a^{Na} in the same skins was 0.43 kΩ^{-1} cm^{-2} (Table 16), and the $\varphi_a^{Na} = 49$ mV. When Cl ions are passive, $\Phi^{Cl} = 0$ for $\varphi = 0$. Then the electrical energy released is 0.84 μ watt cm^{-2} when the conductance value is used and 0.93 μ watt cm^{-2} when the φ_a^{Na} value is used in the calculations. This corresponds to about 3 per cent of the total energy released by the oxidation processes.

If instead the φ_a^{Na} and G_a^{Na} values calculated according to the hypothesis in 2.3.D.c are used, the corresponding values are 1.4-1.6 μ watt cm^{-2} and 6 per cent.

Open-circuited skins. Similar calculations can be made for open-circuited skins. The mean φ_a^{Na} in 13 skins was 76 mV, and the corresponding mean partial conductances were: G_a^{Na} 0.55 and $G^{Cl} = 0.45$ kΩ^{-1} cm^{-2} (Table 6). As, according to the assumptions (see 1.5 and 2.3.A), Φ_a^{Na} and Φ^{Cl} should be equal, which has also been shown experimentally to be approximately true (see Table 17), it is possible to calculate the mean current carried by the Na and Cl ions through the skins. This is found to be 196 $\mu\mu F$ cm^{-2} sec^{-1} or 19 μA cm^{-2}. It will be seen that this value agrees with the current produced by the short-circu-

ited skins). The output of electrical energy in the open-circuited skin may then be estimated as 1.4 μ watt cm^{-2}, or about 5 per cent of the total energy released by oxidation processes.

If the φ_a^{Na} and G_a^{Na} values calculated according to the hypothesis in 2.3.D.c are used, the current carried by actively transported Na ions is 275 $\mu\mu F$ cm^{-2} sec^{-1} and the figures for the electrical energy output 4.8 μwatt cm^{-2} and 15 per cent.

c. ***The "Coulomb efficiency" of the skin.*** If it is assumed that all the electrons transfered for the reduction of oxygen are transported by a redox system like that proposed by Conway (see 2.3.D.a), it is possible to compare the amount of sodium ions transported through the skin with this electron transport. 4 F of electricity are required for the reduction of 16 g oxygen to water. An oxidation rate of 4.8 μl O_2 cm^{-2} hr^{-1} or 1.33 mμl O_2 cm^{-2} sec^{-1} thus requires a transfer of electrons corresponding to 238 $\mu\mu F$ cm^{-2} sec^{-1}. The mean sodium transport, which in short-circuited skins corresponds to a current of 197 $\mu\mu F$ cm^{-2} sec^{-1}, is then seen to be 82 per cent of this value. This percentage corresponds to what Lund and Stapp (1947), employing similar reasoning, call the »Coulomb efficiency» of the skin. Not all of the oxygen can be consumed by the cells which perform the active transport, so these results call for a high efficiency in the redox pump, if such a mechanism does function in the skin. This is no contradiction of the Conway hypothesis, however, since the hypothesis can accommodate a 100 per cent efficiency.

Whether or not the values of G_a^{Na} and φ_a^{Na} obtained from the hypothesis in 2.3.D.c are used makes no difference for short-circuited skins. In the open-circuited skins, however, a »Coulomb efficiency» of 115 per cent is obtained, which is not in conformity with the Conway pump. It should be remarked, however, that the respiration was measured in skins which were effectively short-circuited by the bathing fluid, and the Q_{O_2} values are therefore best compared with the Na flux in short-circuited skins. Further the experimental values vary rather widely.

It is possible that the figure for oxygen consumption is a little

low. If we instead use a Q_{O_2} of 1.08, which can be obtained from the values given by FRANCIS (1934) for summer frogs, and which agrees with the value 0.97 given by PONDER and MACLEOD (1937) for the ventral skin of bull frogs, the oxygen consumption may be estimated to correspond to an electron transfer of 296 $\mu\mu F$ cm^{-2} sec^{-1}. Even then, the sodium transport is 67 per cent of the total electron transfer in short-circuited skins.

It is also possible that the Q_{O_2} value used is high. The temperature in the respiratory experiments was 21.3 C and if a Q_{10} of 1.9 is assumed in this temperature range (FRANCIS 1933), an oxygen consumption of 4.2 μl cm^{-2} hr^{-1} seems more probable at 20 C, the temperature at which the experiments with radioactive isotopes were performed. This value would correspond to a »Coulomb efficiency» of 93 per cent in short-circuited skins.

There are many reasons for believing that the sodium transport is performed by the epithelial cells of the skin. These cells have been shown to have a high oxygen consumption (ERDMAN and SCHMERL 1926), higher than the skin as a whole and higher than other tissues of the frog (cf. ADOLPH 1929) apart from mucosa from the intestinal tract (see Crane 1950). It therefore seems quite probable that most of the oxygen is consumed by the epithelium, and the above figures do not then seem improbable. They may also agree with the results obtained by LUND and STAPP (1947). Owing to their way of calculating, their »Coulomb efficiency» is probably too low (cf. 2.1.a), maybe about half of what it should be. If doubled, it agrees roughly with the »Coulomb efficiency» of the skins in the present investigation.

B. Experiments with mercury compounds.

Mersalyl and $HgCl_2$ influence both active transport of sodium ions and the oxygen uptake of frog skin. However, the effects on these two processes begin at different concentrations, and are not parallel in all respects.

Mersalyl (0.1 mM), for instance, effectively *inhibits* the active transport of sodium ions, while at the same concentration it

increases the respiration by about 50 per cent. Obviously, the inhibition of the active transport is not due to a general toxic effect of the drug on the cells, but seems to be a rather specific action on the sodium pump or processes closely connected with it. Mersalyl can thus be said to reduce the »Coulomb efficiency» of the skin to very low values.

$HgCl_2$ inhibits the sodium pump at a concentration of 0.05 mM (3.1 and LINDERHOLM, unpublished experiments), according to the change in electrical potential across the skin, bathed with Ringer's solutions of the same concentration on either side. At this concentration it does not inhibit the oxygen consumption, and may even stimulate it at a concentration of 0.1 mM. At these concentrations its action is therefore similar to that of mersalyl. At higher concentrations it markedly inhibits respiration. So this substance too seems to have quite a specific effect on the sodium pump.

Mersalyl and $HgCl_2$ are effective in approximately the same molar concentrations, which suggests that the mercury is the active component. Much evidence has been assembled that mercury acts by combining with SH groups in the cells (for ref. see PITTS and SARTORIUS 1950, BEYER 1950). It has been suggested (LEHMAN, BARRACK and LEHMAN 1951) that the dithiol BAL (British antilewisite) breaks the carbon mercury bond of organic mercury diuretics *in vitro*, and that enzymes with more than one SH group in the molecule may combine with mercury, splitting it off from the mercurial diuretic. Such a reaction would explain the delayed effect of mersalyl on frog skin, in contrast to the almost immediate effect of $HgCl_2$. It may also explain the fact that mersalyl does not act on some respiratory enzymes that are evidently inhibited by $HgCl_2$. Differences in permeability and ability to reach the enzymes may also be of importance in this connection. Streptomycin, for instance, was found to act differently on enzymes of animal and bacterial cells, probably owing to permeability differences (UMBREIT and TONHAZY 1949). Further conclusions about the enzymatic reactions should therefore be postponed till more experimental data have been obtained.

It may seem incompatible with the Conway hypothesis that

the inhibition of the sodium pump should be connected with an increased respiration. However, there are many possible ways in which mercury might produce this effect, if we consider its tendency to complex formation and catalytic action. It may possibly change or combine with the specific carrier, making the transport impossible, or it may change the route of the electron transfer in the oxidation processes.

The effect of these small amounts of mercury on the sodium pump conforms with results obtained with regard to active ion transport in other biological objects. MUDGE (1951) has found that $HgCl_2$ and mersalyl in concentrations of 0.01—0.1 mM decrease the ability of rabbit kidney slices to accumulate potassium. INGRAM, PETERS and VISSCHER (1938) showed a marked inhibition of the active transport of ions across the intestinal mucosa by $HgCl_2$ in concentrations of 0.1 mM, and HOLM-JENSEN (1948) has observed a decreased active uptake of sodium ions with *Daphnia Magna* in solutions of 3.2×10^{-7} M $HgCl_2$; these are just some of the relevant papers. The stimulating action of mercury in small concentrations on respiration has been known for some time (cf. SCHADE 1923).

C. Experiments with aminophylline.

Aminophylline increases the respiration, and the ability of the skin to produce an electrical current as well. Its main action on the sodium pump seems to be a decrease of its »internal resistance», as indicated by the high G_a^{Na} values after aminophylline. The active transport potential η_a^{Na} appears to be essentially unchanged and also the relation between the partial conductances of the actively and passively transported ions.

If we try to estimate the mean »Coulomb efficiency» during the action of aminophylline, assuming a mean O_2 consumption of 7.7 μl O_2 cm^{-2} hr^{-1}, or 60 per cent higher than in the controls (cf. Table 14), it is found to be 83 per cent before and 63 per cent during the action of the drug in 4 short-circuited skins. In 2 open-circuited skins the corresponding figures are 92 and 82 per cent. The »Coulomb efficiency» thus seems to decrease in spite of the increased current production by the skin. The overall efficiency also decreases.

If the G_a^{Na} and φ_a^{Na} values obtained from the hypothesis in 2.3.D.c are used, the »Coulomb efficiency» is 104 per cent in the open-circuited skins during the action of the drug.

The action of aminophylline seems to be in many respects similar to that of adrenalin on frog skin. Thus both adrenalin and aminophylline increase the current produced by the skin (cf. USSING and ZERAHN 1951), and the permeability or conductance of the skin (JÖRGENSEN 1947, LINDERHOLM 1950). Adrenalin might also be expected to increase the respiration (cf. AHLGREN 1926, v. EULER 1930, and LUNDHOLM 1949). Adrenalin, however, decreases the active sodium transport potential; and neurohypophyseal extract, which increases the partial conductance of Na ions while leaving the active sodium transport potential constant (USSING and ZERAHN 1951), may therefore have an action more similar to that of aminophylline. The action of caffeine may also be similar to that of aminophylline (UHLENBRUCK 1924, LINDERHOLM, unpublished experiments).

D. Experiments in which a change in pH was induced.

A decrease in pH inhibits both the potential and the oxygen consumption of the skin. If the effect is not specific to CO_2, a decrease in pH seems to influence the sodium pump much more than the respiration. When CO_2 was bubbled through the solutions to produce a pH of about 6.5, this effectively decreased the φ_a^{Na} to very low values or to zero, while the respiration was inhibited only about 30 per cent by a similar pH change induced by adding HCl. It seems quite probable that the change in pH affects the carrier complex in some way, as is indicated by the low G_a^{Na} value. For a good discussion of possible explanations the reader is referred to a paper by USSING (1949 b).

An alkaline medium is known to increase the active transport of sodium (USSING 1949 and HUF et al. 1951). It also seems to increase the respiration. Whether a high pH increases the active transport potential or increases the sodium transport like aminophylline by increasing the conductance, it is impossible to say from the present experiments.

5.2. Possible relations between the effects on frog skin and on kidney of the diuretics studied.

It seems to be a generally accepted view that mercurial and xanthine diuretics excert their main action on the renal tubuli by decreasing their ability to reabsorb electrolytes and water (for references see PITTS and SARTORIUS 1950, DICKER 1951, BEYER 1950). The reabsorption of sodium and chloride ions in the tubuli, which with a normal glomerular filtration rate corresponds to more than one kg of NaCl per day in a human subject, is generally ascribed to an active sodium reabsorption (see WESTON, ANSLOW and SMITH 1948, and SMITH 1951).

Actually, it may just as well be ascribed to an active reabsorption of chloride. HOGBEN (1950) has given evidence for the occurence of active transport of Cl ions in the gastric mucosa of frogs. Some measurements of the electrical potential across the epithelial layer of a tubule in the kidney of *Necturus*, with Ringer's solution on both sides of the wall (WILBRANDT 1938), showed that the lumen of the tubule was negative in the proximal and positive in the intermediary part with respect to the outside. This observation might be accounted for by an active reabsorption of sodium ions in the proximal part of this tubule, and of chloride ions in the intermediary part. No reports on potential measurements on tubules in mammals have been found. However, is seems probable that an important part of the reabsorption of salt in the kidney tubule is due to an active transport of sodium ions. Since such a transport seems to be a primary function of animal cells, the mechanism might be expected to be similar in the kidney and in frog skin.

a. ***Mercury compounds.*** The inhibition of the active transport of sodium ions in frog skin by mersalyl (and $HgCl_2$) suggests that a similar inhibition of a »sodium pump» in the kidney is a main feature in the genesis of mercurial diuresis.

The concentration of the drug effective for frog skin is rather high, compared with the blood concentration after the administration of an ordinary dose of a mercurial diuretic. The mercury, however, is fixed in the kidney (WESTON, GROSSMANN, LEHMAN, UHLMANN, HALPERIN and LEITER 1951) and its concen-

tration is high enough to inhibit the succinic dehydrogenase activity in the kidney cortex of an animal (HANDLEY 1949) after an intravenous injection. There is no simultaneous inhibition of this enzyme in heart and liver tissue from the same animal. When the diuretic was added *in vitro* to these tissues at a concentration of 10^{-5} M, inhibition occurred. This concentration range corresponds well with the doses which are effective for frog skin, of $HgCl_2$ at least. Mersalyl seems to require a slightly higher concentration.

In man, even high doses of mercurial diuretics never cause an inhibition of the reabsorption of salt by more than about 20—40 per cent of the amount filtered (PITTS and DUGGAN 1950, WESTON, GROSSMAN and LEITER 1951). This may indicate that there is another mechanism than the »sodium pump» for the active transport of ions in the kidney.

b. ***Aminophylline.*** The action of aminophylline on frog skin does not appear to offer an explanation of its diuretic effect on the kidney, unlike that of mersalyl. On the contrary, one would rather expect an increased reabsorption in the tubule as a result of the action of aminophylline. However, it is possible to advance some reasons why aminophylline should decrease the reabsorption of electrolytes in the renal tubule, in spite of the fact that it increases the active transport of NaCl through frog skin.

It may be that aminophylline increases the permeability of the tubule to passive ions relatively more than it decreases the »internal resistance» of the sodium pump, though such an effect was not very marked in frog skin. Then the effective transport potential for sodium ions would decrease, cf. 1.6.a. It should also be borne in mind that the renal tubule is not so simple a system as the frog skin in the present experiments. When there has been some reabsorption from the tubules the concentration of other ions than Na^+ and Cl^- will become important. If only Na ions are actively transported, the other ions will tend to decrease the net transfer of NaCl, cf. 2.3.D.e p. 87.

Finally, we cannot exclude the possibility that aminophylline may act on some active transport mechanism other than a »sodium pump», if such a »pump» exists in the kidney.

c. ***Acidifying agents.*** A low pH effectively inhibits the »sodium pump« of frog skin. Acidifying agents are used as diuretics, and they are also known to potentiale the action of mercurial diuretics. It might therefore be expected that a low pH in the plasma and in the primary urine could explain this action. HILTON (1951) found, however, that in normal subjects potentiation of a mercurial diuretic by ammonium chloride was independent of alteration in the plasma acid base balance. In dogs rendered acidotic by inhalation of 7 per cent CO_2 there is no enhanced activity from the injection of a mercurial diuretic (AXELROD, CAPPS, and PITTS 1950). Consequently, the action of acidifying agents may be more complicated.

Summary.

Chap. 1. The theoretical part deals with active transport phenomena as they may occur in living cells or tissues, especially with regard to electrolytes. Active transport is defined, and the force involved in the active transport is expressed as a potential term, additive to potentials due to other forces which may act on an ion or an uncharged molecule.

The case when a single electrolyte is present on both sides of the membrane where active transport occurs is examined, and formulas are derived which make it possible to calculate the partial electrical conductance of the ions when their fluxes are measured by means of radioactive isotopes. Formulas are also derived for the calculation of active transport potentials and active transport of salt. Active transport of water is expressed in a similar way to that of electrolytes.

Chap. 2. Experiments were carried out with the combined use of electrical methods for measuring conductance and electrical potential and tracer methods for measuring ionic fluxes through isolated frog skin. Equations derived in the theoretical part were used in the treatment of the data obtained. The active transport potential of sodium, the partial conductances of the Na and Cl ions — the sum of which was found to agree very well with the electrically measured total conductance — and the transport of salt through the skin were determined.

The results indicate that only Na ions are transported actively inwards through the frog skin under the present experimental conditions, while Cl ions behave as passive ions. Frog skin with Ringer's solution of the same concentration on both sides has an active sodium transport potential of about 75 mV, a total conductance of about 1 k Ω^{-1} cm^{-2} and a ratio between the partial conductance of Na and Cl ions of 1.5. The active transport of NaCl amounts to about 180 $\mu\mu$mol cm^{-2} sec^{-1}. The »short-circuited skin» produces a mean electric current of 19 μA cm^{-2} which seems to correspond to the net flux of Na ions.

Frog skin with a dilute Ringer's solution on the outside has a much lower conductance (the more dilute the solution the lower it is), the ratio between the partial conductances of Na and Cl ions seems to be higher than in skins with Ringer's solution of the same concentration on both sides, and the active transport potential also increases. At high dilutions this should, according to hypothesis, approach the actual transport potential of the postulated »sodium pump» which has been estimated of about 150—170 mV. The highest transport potential found in a skin with a 7 mM NaCl solution on the outside was 138 mV, which allows the frog skin to balance a concentration difference of more then 1:10. This ability is probably more pronounced in the intact frog.

It was found that of different mechanisms which have been proposed for the active transport of ions, the »redox pump» described by CONWAY agreed best with the experimental data.

The earlier observation of a characteristic potential-log (concentration) curve, which was explained on the basis of the Teorell-Meyer-Sievers theory for charged membranes, is discussed. A new hypothesis is advanced, in which a specific sodium carrier in the frog skin is postulated to explain these phenomena. This hypothesis is found to be in some respects a better explanation of experimental findings, and makes it possible to calculate the partial conductance of Na ions in the postulated carrier system.

Chap. 3. a. The effects of mersalyl on frog skin were studied by the methods described in Chap. 2. It was found that mersalyl (0.1 mM) markedly inhibits the active transport of salt, reducing the active transport potential to low values. The conductance of the skin usually increases after the drug has acted for some time. The partial conductance of Na ions in the postulated carrier system usually decreases a great deal.

b. The effects of aminophylline were likewise studied. It was found that this drug does not essentially change the active transport potential of the Na ions, but it increases the partial conductances of these ions in the carrier system (decreases the internal resistance of the »sodium pump»), and also generally

increases the total conductance and permeability of frog skin to electrolytes.

c. The effects of pH changes induced by CO_2 were studied, and it was found that a low pH decreases the active transport potential of the »sodium pump» and the partial conductance of Na ions in the carrier system, as well as the total conductance of the skin.

Chap. 4. The oxygen uptake of frog skin was studied under experimental conditions similar to those of the previous experiments. It was found that both mersalyl and aminophylline increase and $HgCl_2$ decreases the oxygen uptake. A low pH decreases the oxygen uptake while a high pH seems to increase it.

Chap. 5. The results obtained from the experiments with radioactive isotopes are considered together with those from measurements of the oxygen uptake of frog skin. It is found that the minimum energy required for the transport of sodium ions in »normal» skins is at least 3—5 per cent of the total energy released by oxidation processes. The quantity of electricity transferred with the Na ions in the active transport is found to be 70 to 90 per cent of the quantity of electricity transferred by electrons in the oxidation processes. This percentage is called the »Coulomb efficiency» of the skin. The high figure requires a »Coulomb efficiency» of the »sodium pump» of nearly 100 per cent. The »Coulomb efficiency», and overall efficiency are markedly decreased by mersalyl, $HgCl_2$ and a low pH, and decreases slightly even after aminophylline.

Some possible relations between the effect of the diuretic drugs studied on frog skin and the effect of these drugs on the kidney are discussed. The depressing action of mersalyl on the »sodium pump» offers a good explanation of the decreased reabsorption of salt in the renal tubule, while the action of aminophylline is more difficult to explain. The effect of the low pH on frog skin probably has little to do with the effect of acidifying agents on diuresis.

Acknowledgements.

The investigation was aided by grants from the Regnell fund of the Medical Faculty in Uppsala and from the Swedish State Fund for Promotion of Medical Research. Miss Ebon Hellström has given valuable technical assistance and Miss Maj Uhrström prepared the figures. Mr R. Stedman revised the English text.

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